Caractérisation d'espèces bioindicatrices pour la surveillance des activités minières et la gestion de l'environnement en milieu récifal et lagonaire : application au lagon de Nouvelle-Calédonie

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Caractérisation d'espèces bioindicatrices pour la surveillance des activités minières et la gestion de l'environnement en milieu récifal et lagonaire : application au lagon de Nouvelle-Calédonie

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« Nous ne sommes que les gardiens de la terre de nos enfants»

Paul Sihazé, Grand Chef du district de Wetr, Lifou

« Vagabonder à la surface des océans est souvent source de sérénité et, parfois, permet de tutoyer ses rêves. S'y immerger, c'est s'ouvrir à son observation et à sa compréhension »

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A mes parents,

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- Warnau M, **Hédouin L**, Teyssié J-L & Fichez R. Use of radiotracer techniques to characterize experimentally the bioindicative value of tropical bivalves.

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- Hédouin L, Pringault O, Bustamante P., Fichez R & Warnau M. Ni Bioaccumulation in tropical oysters and clams: Effect of dissolved Ni concentration.

- Hédouin L, Gomez Batista M, Metian M, Borne V, Cotret O, Teyssié J-L & Warnau M. Influence of ambient dissolved concentration of metals on their bioaccumulation in three marine bivalves from New Caledonia.

- Metian M, **Hédouin L**, Teyssié J- L, Bustamante P. &Warnau M. Bioaccumulation of selected heavy metals in the king scallop (Pecten maximus) and the black scallop (Chlamys varia) : sea water and food exposures.

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International Conference On Isotopes in Environmental Studies - Aquatic Forum 2004, IAEA, Monaco, 25-29 Octobre 2004

- Metian M, **Hédouin L**, Giron E, Buschiazzo E, Borne V, Cotret O, Teyssié J-L, Fowler SW & Warnau M. Bioaccumulation of selected heavy metal radiotracers in the brown alga Lobophora Variegata (Lamouroux).

- Hédouin L, Giron E, Buschiazzo E, Gomez Batista M, Metian M, Cotret O, Teyssié JL, Fowler SW & Warnau M. Influence of ambient dissolved metal concentration on bioaccumulation in two tropical oysters: A radiotracer study.

- Hédouin L, Metian M, Cotret O, Teyssié J-L, Paganelli J, Fowler SW, Fichez R & M Warnau. Delineation of heavy metal uptake pathways (seawater, food and sediment) in tropical marine bivalves using metal radiotracers.

37th CIESM (Commission Internationale pour l'Exploration Scientifique de la mer Méditerranée), Barcelone, Espagne, 7-11 Juin 2004

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INTRODUCTION GÉNÉRALE

La Terre est souvent appelée « planète bleue » en raison de l'étendue de ses océans. Ces derniers ont permis l'apparition de la vie sous sa forme la plus primitive, il y a un peu moins de 4 milliards d'années. Au cours des temps géologiques, la vie sur la Terre s'est développée, et l'*Homo sapiens* « homme moderne » est apparu. Pour tous les organismes vivants, la nature est un milieu de vie. Néanmoins, la richesse de la nature est souvent considérée par l'Homme comme une ressource naturelle pour la vie quotidienne et les communautés humaines ont joué un rôle majeur en terme d'évolution de la biodiversité, considéré comme le « reflet » de nos relations avec les autres organismes vivants.

En ce nouveau millénaire, certains se demanderont peut-être si l'Homme a pris soin de préserver cet « écosystème géant », appelé Terre. Un triste constat s'impose : l'accès à l'eau est aujourd'hui considéré comme un accès à la richesse, l'augmentation continue des rejets des gaz à effet de serre risque dans quelques années de créer un réchauffement climatique aux conséquences encore mal appréhendées, l'agriculture basée sur la culture d'organismes génétiquement modifiés (O.G.M) semble en passe de supplanter notre agriculture traditionnelle sans avoir totalement établi l'impact de ces O.G.M sur la santé humaine et sur l'environnement, l'industrie nucléaire est maintenant confronté au démantèlement des centrales en fin de vie. Tous ces problèmes illustrent combien l'espèce humaine peut modifier l'équilibre de la biosphère. Le rapport du Programme National des Nations Unies (PNUE 1980) ainsi que bien d'autres études soulignent l'état catastrophique de notre environnement et l'urgence dans laquelle nous nous trouvons. L'environnement marin, atmosphérique et terrestre est aujourd'hui atteint et certains parlent d'un « mal de l'environnement ». L'homme a dépassé les limites de la « *capacité de charge* » de la planète (Soule 1981) et l'érosion de la biodiversité est un fait marquant et alarmant de ces dernières décennies. Selon l'avis des biologistes, une extinction de masse est en train de se produire (Wilcox 1988; Sodhi et al. 2004). Chaque année, entre 17 000 et 100000 espèces disparaissent de notre planète. D'ici l'an 2100, la déforestation aura éliminé pratiquement toutes les forêts tropicales, à l'exception des zones protégées (Houghton 1990) et les récifs coralliens sources de diversité marine sont tout aussi menacés (Briggs 2006). Pour la plupart des scientifiques, les activités humaines sont responsables non seulement de la modification et de la perturbation des équilibres, mais aussi de la destruction d'écosystèmes abritant certaines espèces végétales et animales (Swanson 1997). Deux raisons principales permettent d'expliquer la dégradation de notre environnement : la croissance économique et la croissance démographique. De l'ère industrielle à nos jours, la population mondiale a augmenté d'un facteur six, entraînant par la

même occasion, une augmentation de la demande de ressources et une augmentation de la contamination. En effet, la croissance économique caractérisée par une forte expansion des industries a engendré l'arrivée dans l'environnement d'un grand nombre de substances minérales ou organiques, non biodégradables et parfois toxiques, dont les métaux.

I. GENERALITES

I.1. CONTAMINATION ET POLLUTION

I.1.1. Définition

L'étymologie du terme « polluer » signifie profaner, souiller, salir, dégrader. D'usage courant à l'heure actuelle, le terme de pollution qualifie généralement toute action susceptible de porter atteinte à l'équilibre écologique et de menacer la vie (animale, végétale et humaine) sur la Terre. Kinne (1968) la définit comme « *les activités humaines qui ont des effets négatifs sur la santé, les ressources, le bien-être et les écosystèmes* ». Or, il convient de différencier la contamination de la pollution, les contaminants des polluants (Warnau 1996). Les métaux présents dans l'environnement sont qualifiés de « contaminants » lorsqu'ils sont présents en concentration supérieures au bruit de fond naturel en l'élément considéré (GESAMP 1984). Ces contaminants peuvent devenir toxiques s'ils se retrouvent en quantités suffisantes dans les organismes vivants. Ces « contaminants » seront qualifiés de « polluants » lorsqu'ils sont d'origine anthropique, et qu'il en résulte des effets dommageables pour le milieu, les organismes, la santé humaine ou les activités humaines (GESAMP 1984).

I.1.2. Historique

La contamination métallique ne constitue en aucun cas un problème récent ou un phénomène épisodique. Ses origines remontent aux premières civilisations. A cette époque, les métaux étaient utilisés pour la vie de tous les jours par les populations : l'or, l'argent, le cuivre servaient à la fabrication des pièces de monnaies; le plomb dans les canalisations et les récipients de cuisine. Ces derniers furent responsables de l'intoxication par le plomb des populations, appelée saturnisme. Bien des siècles se sont écoulés depuis ces lointaines civilisations, et la quantité de plomb rejetée dans l'environnement n'a cessé d'augmenter, notamment pendant la révolution industrielle et à la suite de son utilisation en tant qu'additif dans les carburants depuis les années 1920. Néanmoins, le plomb est apparu comme un réel

danger pour la santé humaine et l'environnement, principalement depuis les années 1970. Dès lors, des efforts ont été réalisés pour diminuer son utilisation, notamment grâce à la fabrication d'essence sans plomb réduisant ainsi considérablement les émissions de ce métal dans l'atmosphère.

Le rejet intempestif de métaux dans l'environnement pose toujours des problèmes majeurs, accentués selon la toxicité du métal. Considéré comme un des métaux les plus toxiques, le mercure a été la cause d'une des plus grandes tragédies de pollution métallique : l'affaire de Minamata au Japon. La population locale consommait de grandes quantités de poissons et coquillages provenant de la baie Minamata, dans lesquels se concentrait le mercure rejeté par une usine. Alors que le méthyl-mercure n'excédait généralement pas plus de 0,1 $\mu g\ l^{-1}$ dans l'eau de mer, des concentrations de l'ordre de 50 $\mu g\ g^{-1}$ ont été mesurées dans certains poissons de cette baie. Dans les années 1950 et 1960, 150 tonnes de mercure y ont ainsi été déversées et les taux de mercure trouvés dans les poissons contaminés étaient 500 000 fois supérieurs à ceux des eaux de la baie (Ui 1971). La tragédie de Minamata en 1956 a ainsi provoqué l'intoxication de 2000 personnes (Tsubaki & Irukayama 1977).

Au-delà des effets spectaculaires des marées noires (e.g. l'accident du Prestige en 2002) lié à leur impact visuel, les contaminants métalliques qui infiltrent le milieu marin sont invisibles à l'œil, et pourtant forts dangereux de part leur puissante toxicité. La pollution métallique est dite « insidieuse et sournoise ». Dans l'histoire des pollutions, l'effet visuel des marées noires associé au caractère toxique de métaux (e.g., Hg) ont fait prendre conscience, non seulement au grand public mais aussi aux décideurs, de la gravité des conséquences pouvant résulter de la contamination, accidentelle ou chronique, de l'environnement.

I.2. NAISSANCE DU DROIT INTERNATIONAL DE L'ENVIRONNEMENT ET DE L'ECOTOXICOLOGIE

I.2.1. Naissance de l'écotoxicologie : un besoin

Née dans les années 60, à l'instigation du professeur Truhaut, l'écotoxicologie est une science pluridisciplinaire. Elle allie chimie, toxicologie, écologie, environnement et biologie, et permet l'étude du devenir des contaminants dans l'environnement. Au départ, cette science nouvelle s'intéressait essentiellement à la toxicité directe des contaminants sur l'homme, mais son parcours a évolué vers l'étude des effets des contaminants sur toutes les formes de vie. Truhaut (1977) la définit comme « *la branche de la toxicologie qui étudie les effets toxiques*

provoqués par les substances naturelles ou les polluants d'origine synthétique sur les constituants des écosystèmes animaux, y compris l'Homme, végétaux et micro-organismes, dans un contexte intégré ». De nombreuses définitions existent pour parler de l'écotoxicologie, mais celle de Rand & Petrocelli (1985) sur l'écotoxicologie des écosystèmes aquatiques correspond peut-être le plus à l'approche utilisée : *« la toxicologie aquatique est aussi concernée par les concentrations et les quantités de produits chimiques qui peuvent être présentes dans l'eau, les sédiments et les productions aquatiques; elle inclut donc l'étude du transport, de la distribution, de la transformation et du devenir ultime des substances dans l'environnement aquatique »*. Lors de l'évolution de l'écotoxicologie, il est apparu important de renforcer les études sur les effets des mécanismes écologiques de la pollution des écosystèmes (Koeman 1982; Cairns 1988). L'écotoxicologie est une intégration de l'écologie et de la toxicologie (Chapman 1995, 2002) (Fig. 1).

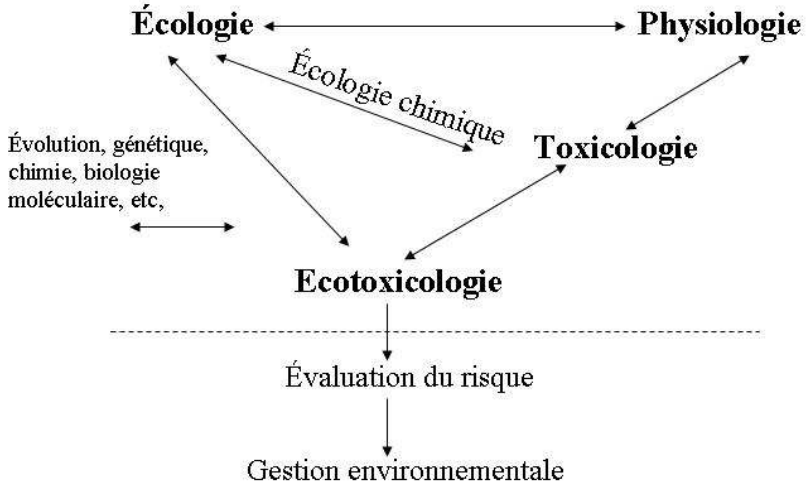

Figure 1. Relations de l'écotoxicologie avec les autres disciplines, le risque environnemental et la gestion environnementale (Sources : Forbes & Forbes 1997).

Si l'écotoxicologie permet de s'intéresser à la question du devenir et de l'impact de contaminants sur les écosystèmes au niveau scientifique, il est apparu important d'appréhender juridiquement la question des pollutions. Les contaminants ne connaissent pas de frontière. Ainsi pour surveiller les contaminants dans l'environnement marin, il ne faut pas s'arrêter aux frontières des états. Les problèmes juridiques de l'impact des contaminants sur l'environnement doivent être analysés à une échelle mondiale. De cette vision globale des problèmes de l'environnement est né le droit international de l'environnement.

I.2.2. Droit international de l'environnement

La prise de conscience écologique des années 60-70 correspond à la naissance du droit international de l'environnement, apparue comme une nécessité afin de protéger l'environnement selon des règles juridiques internationales. L'année 1972 peut-être considérée comme une année décisive pour l'environnement avec l'organisation de la première Conférence Internationale sur l'Environnement à Stockholm en 1972, donnant le droit « *à un environnement de qualité permettant de vivre dans la dignité et le bien-être* ». Cette conférence fût le premier pas à franchir pour considérer les problèmes environnementaux à une dimension internationale *: « penser à l'échelle mondiale et agir à l'échelle locale »*. Les années 80 furent marquées par la question de la relation possible entre le développement et l'environnement. La publication du « Rapport Bruntland : Notre avenir commun » en 1987 fit apparaître la notion de « *substainable development* » traduit en français par « *développement durable* », et considéré comme un développement possible pour les générations présentes sans compromettre les ressources pour les générations futures (Bruntland 1987). Le Sommet de la Terre à Rio en 1992 réaffirma cette volonté de mettre en place un développement durable; et de nombreuses mesures et conventions furent signées (Action 21, Convention sur la Diversité Biologique CDB, Convention Cadre des Nations Unies sur les Changements Climatiques CCNUCC). Le début du nouveau millénaire a été marqué par la Conférence Internationale de Kyoto 2002, et par le refus des États-unis de ratifier le Protocole de Kyoto, visant à réduire la production des gaz à effet de serre.

II. LES METAUX ET LES ORGANISMES MARINS

II.1. QU'EST CE QU'UN METAL ?

II.1.1. Définition

Un métal est un élément chimique capable de former des liaisons métalliques ou ioniques. Les métaux forment un des trois groupes d'éléments distingués par leurs propriétés d'ionisation et de liaison chimique, les deux autres groupes étant les métalloïdes et les non-métaux. Les métaux occupent globalement les quatorze premières colonnes du tableau de classification périodique (Fig. 1). Ce sont en général des solides cristallins, néanmoins le mercure existe à l'état liquide dans les conditions normales (20 °C sous pression atmosphérique). Ils ont des propriétés physiques intéressantes : malléables et ductiles, bon conducteur de chaleur et

d'électricité, possibilité d'alliages. Les métaux sont des contaminants non conservatifs, non dégradables, ni biologiquement, ni physico-chimiquement (Clark 1989).

Classification Périodique des éléments chimiques

Couche	Principales colonnes I	II	III	IV	V	VI	VII	VIII
K	$^{1}_{1}H$ 1,01							$^{4}_{2}He$ 4,00
L	$^{7}_{3}Li$ 6,94	$^{9}_{4}Be$ 9,01	$^{11}_{5}B$ 10,81	$^{12}_{6}C$ 12,01	$^{14}_{7}N$ 14,01	$^{16}_{8}O$ 16,00	$^{19}_{9}F$ 19,00	$^{20}_{10}Ne$ 20,18
M	$^{23}_{11}Na$ 22,99	$^{24}_{12}Mg$ 24,31	$^{27}_{13}Al$ 26,98	$^{28}_{14}Si$ 28,09	$^{31}_{15}P$ 30,97	$^{32}_{16}S$ 32,07	$^{35}_{17}Cl$ 35,45	$^{40}_{18}Ar$ 39,95
N	$^{39}_{19}K$ 39,10	$^{40}_{20}Ca$ 40,08	$^{45}_{21}Sc$ 44,96	$^{48}_{22}Ti$ 47,87	$^{51}_{23}V$ 50,94	$^{52}_{24}Cr$ 52,00	$^{55}_{25}Mn$ 54,94	$^{56}_{26}Fe$ 55,85	$^{59}_{27}Co$ 58,93	$^{58}_{28}Ni$ 58,69	$^{63}_{29}Cu$ 63,55	$^{64}_{30}Zn$ 65,41	$^{69}_{31}Ga$ 69,72	$^{74}_{32}Ge$ 72,64	$^{75}_{33}As$ 74,92	$^{80}_{34}Se$ 78,96	$^{79}_{35}Br$ 79,90	$^{84}_{36}Kr$ 83,80
O	$^{85}_{37}Rb$ 85,47	$^{88}_{38}Sr$ 87,62	$^{89}_{39}Y$ 88,91	$^{90}_{40}Zr$ 91,22	$^{93}_{41}Nb$ 92,91	$^{98}_{42}Mo$ 95,94	^{43}Tc [98]	$^{102}_{44}Ru$ 101,07	$^{103}_{45}Rh$ 102,91	$^{106}_{46}Pd$ 106,42	$^{107}_{47}Ag$ 107,87	$^{114}_{48}Cd$ 112,41	$^{115}_{49}In$ 114,82	$^{120}_{50}Sn$ 118,71	$^{121}_{51}Sb$ 121,76	$^{128}_{52}Te$ 127,60	$^{127}_{53}I$ 126,90	$^{129}_{54}Xe$ 131,29
P	$^{133}_{55}Cs$ 132,91	$^{138}_{56}Ba$ 137,33	$^{175}_{71}Lu$ 174,97	$^{180}_{72}Hf$ 178,49	$^{181}_{73}Ta$ 180,95	$^{184}_{74}W$ 183,84	$^{185}_{75}Re$ 186,21	$^{192}_{76}Os$ 190,23	$^{193}_{77}Ir$ 192,22	$^{195}_{78}Pt$ 195,08	$^{197}_{79}Au$ 196,97	$^{202}_{80}Hg$ 200,59	$^{205}_{81}Tl$ 204,38	$^{208}_{82}Pb$ 207,2	$^{209}_{83}Bi$ 208,98	$^{210}_{84}Po$ [209]	$^{218}_{85}As$ [210]	$^{222}_{86}Rn$ [222]
Q	$^{223}_{87}Fr$ [223]	$^{226}_{88}Ra$ [226]	^{103}Lr [262]	^{104}Rf [261]	^{105}Db [262]	^{106}Sg [266]	^{107}Bh [264]	^{108}Hs [269]	^{109}Mt [268]	^{110}Ds [268]								

Nombre de masse de l'isotope le plus abondant :A — Numéro atomique :Z — $^{A}_{Z}X_{M}$ — M: Masse molaire atomique en (g/mol) du mélange isotopique naturel

57 à 71 lanthanides	$^{139}_{57}La$ 138,91	$^{140}_{58}Ce$ 140,12	$^{141}_{59}Pr$ 140,91	$^{144}_{60}Nd$ 144,24	^{61}Pm [145]	$^{152}_{62}Sm$ 150,36	$^{153}_{63}Eu$ 151,96	$^{158}_{64}Gd$ 157,25	$^{159}_{65}Tb$ 158,93	$^{162}_{66}Dy$ 162,50	$^{165}_{67}Ho$ 164,93	$^{166}_{68}Er$ 167,26	$^{169}_{69}Tm$ 168,93	$^{174}_{70}Yb$ 173,04
89 à 103 actinides	$^{227}_{89}Ac$ [227]	$^{232}_{90}Th$ 232,04	$^{231}_{91}Pa$ 231,04	$^{238}_{92}U$ 238,03	$^{237}_{93}Np$ [237]	$^{239}_{94}Pu$ [244]	^{95}Am [243]	^{95}Cm [247]	^{97}Bk [247]	^{98}Cf [251]	^{99}Es [252]	^{100}Fm [257]	^{101}Md [258]	^{102}No [259]

Classe A — Classe B — Frontière — Cu^{2+} est un ion métallique frontière, Cu^{+} est un classe B; Pb^{2+} est un ion métallique frontière, Pb^{IV} est un classe B

Tableau 1. Tableau de la classification périodique des éléments montrant la séparation des ions métalliques et métalloïdes en classe A, B et « frontière » (Nieboer & Richardson 1980; Rainbow 1997b).

Les termes « métal lourd » ou « métal trace » sont souvent employés pour désigner un métal. Le terme de « métal trace » insiste sur la présence en quantité très faible des éléments aux propriétés métalliques dans l'environnement, à l'état de « trace » (de l'ordre du µg g^{-1} ou inférieur). Cependant, ce n'est pas toujours le cas. Ainsi, de nombreux auteurs ont privilégié l'utilisation du terme « métal lourd », désignant ainsi les éléments du tableau périodique ayant des propriétés d'éléments métalliques ou semi-métallique et dont la masse atomique est supérieure à 44 (Warnau 1996) ou la masse volumique supérieure à 5 g cm^{-3} (Lapedes 1974). Cette classification en « métal lourd » est discutée. Certains métaux ne sont pas forcément « lourd » (e.g., Al dont la masse volumique est 2,7 g cm^{-3}) et d'autres éléments toxiques ne sont pas forcément des « métaux », (e.g. As qui est un métalloïde et appartient aux éléments semi-conducteurs). Actuellement, un autre schéma de classification des métaux basé sur l'affinité des ions métalliques (ions métalliques de classe A, B et frontière) tend à être utilisé (Rainbow 1997b). Les ions métalliques classe A présentent une forte affinité pour les ligands contenant de l'oxygène (e.g. groupes fonctionnels de types PO_4, CO, COOH) alors que les ions métalliques classe B présentent une forte affinité pour les ligands contenant du soufre -S–

S-, -SH, -SR, -NH_2-. Les ions métalliques classe A forment des complexes plus stables selon la tendance O > N > S, la tendance inverse est observé pour les ions métalliques classe B : S > N > O (Nieboer & Richardson 1980; Rainbow 1997b) (Fig. 1). La chimie des métaux apparaît en effet comme un paramètre important à prendre en compte pour une meilleure compréhension des processus de bioaccumulation des métaux dans les organismes.

II.2. SOURCES DE METAUX ET METALLOÏDES DANS L'ENVIRONNEMENT

Naturellement présents dans la biosphère, les métaux suivent des cycles biogéochimiques qui amènent à une distribution hétérogène de leurs concentrations à la surface du globe (Garret 2000). Les métaux arrivent dans les écosystèmes aquatiques par des apports telluriques naturels, comme l'érosion chimique et mécanique des sols, le transport de particules ou débris rocheux par les cours d'eau, effluents ou précipitations. Les métaux peuvent également être transportés vers l'Océan par des apports atmosphériques (aérosols, poussières, volcanisme), par les eaux souterraines, par l'hydrothermalisme (Bryan 1984; Laws 1993; Nisbet & Fowler 1995). A ces apports naturels s'ajoutent des apports anthropiques issus des activités domestiques, industrielles, militaires, minières et agricoles tels que les rejets accidentels, les rejets des sous-produits des industries et des mines, les eaux de ruissellements (Laws 1993) (Tab. 2).

Éléments	Eau	Air	Sol
As	41	19	82
Cd	9,4	7,6	22
Cr	142	30	896
Cu	112	35	954
Hg	4,6	3,6	8.3
Ni	113	56	325
Pb	138	332	796
Zn	226	132	1372

Tableau 2. Quantités globales de quelques éléments déversés dans l'environnement (1000 tonnes métriques/an) (sources : Nriagu & Pacyna 1988).

Sous l'effet du développement des activités anthropiques, les apports métalliques dans les milieux aquatiques ne cessent d'augmenter. Environ 70% de la population humaine vit près des côtes (Gray 1991) et les zones estuariennes sont des zones propices à l'implantation d'industries de part les facilités de commerce, de circulation, d'élimination des déchets dont elles disposent. Ces environnements subissent donc d'importants apports en contaminants, qui s'accumulent à terme dans les sédiments (Bryan 1980; Gray 1991).

II.3. TOXICITE OU ESSENTIALITE

Constituants naturels de l'écorce terrestre, certains métaux sont indispensables aux organismes vivants (Co, Cu, Fe, Mn, Ni, Zn), au même titre que les sels nutritifs (nitrates, phosphates ou silicates). Les métaux à caractère essentiel jouent un rôle important dans les processus métaboliques. Par exemple, le Cu associé à l'hémocyanine joue un rôle dans le transport d'oxygène de nombreux mollusques et crustacés; le Co est un des constitutif de la vitamine B12, le Zn est un cofacteur des ADN et ARN polymérases, de l'anhydrase carbonique, de certaines déshydrogénases et phosphatases alcalines ; le Ni est un cofacteur des hydrogénases (Bowen 1966).

Ainsi, si les exigences cellulaires en éléments essentiels sont satisfaites, alors les organismes grandissent et se développent de façon optimale. Par contre, si ces éléments sont présents en quantités insuffisantes ou en excès par rapport aux besoins de l'organisme, alors des problèmes de carences ou de toxicité apparaissent. Entre ces deux cas extrêmes, il existe pour les éléments essentiels une « *fenêtre d'essentialité* » (Hopkin 1989) ou une « *gamme de concentration optimale pour les éléments essentiels* » (Van Assche et al. 1997) (Fig. 2).

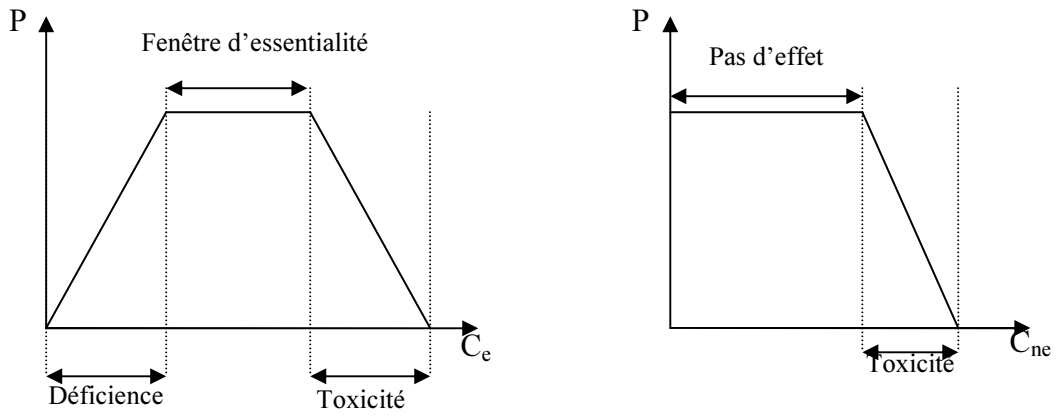

Figure 2. Relation entre la performance (P) (croissance, fécondité, survie) et les concentrations en éléments essentiels C_e ou non essentiels C_{ne} pour les organismes (Source : Hopkin 1989).

Au-delà d'une concentration seuil, des effets toxiques peuvent apparaître pour la faune et la flore (Rainbow 2002). Cette notion de dose est fondamentale, car la plupart des éléments ne sont toxiques qu'à partir d'une certaine dose. La toxicité est défini comme « *relative property of a chemical that concerns its potential harmful effect on a living organism* » (Rand & Petrocelli 1985), qui se traduit par « *la propriété relative d'un composé chimique qui se rapporte à son potentiel effet nocif sur un organisme vivant* ». La toxicité dépend donc de la concentration du métal, de la durée de l'exposition, de la forme physico-chimique du métal dans l'eau (spéciation), de la présence d'autres métaux et de la sensibilité des espèces aux métaux (Meyer 2002). La toxicité des métaux est aussi directement liée à leur accumulation par les organismes, à la réactivité de ces métaux vis-à-vis de molécules d'importance biologique, et à l'aptitude des organismes à métaboliser ou à détoxiquer les métaux « réactifs ».

Certains métaux sont considérés comme non essentiels (Hg, Cd, Pb), car ils n'ont aucune fonction biologique reconnue. Des effets néfastes interviennent quand les concentrations environnementales dépassent un certain seuil à partir duquel les processus de régulation homéostatiques sont saturés. Suite à l'exposition métallique des organismes, des effets létaux ou sub-létaux peuvent apparaître, plus ou moins dangereux, et entraîner des changements morphologiques, des effets inhibiteurs ou des changements de comportements. La détermination de la DL_{50} et de la CL_{50} (dose létale et concentration létale, provoquant 50% de

mortalité au bout d'un temps donné d'exposition sur une espèce donnée) permet d'évaluer la toxicité des métaux vis-à-vis des organismes.

Si la toxicité d'un contaminant prend son origine à l'échelle subcellulaire, les effets d'une contamination peuvent se ressentir à tous les niveaux d'organisation biologique, depuis le niveau subcellulaire jusqu'au niveau écosystémique (Peakall 1992). Les métaux sont d'autant plus dangereux qu'ils peuvent être accumulés dans les organismes (bioaccumulation) et ainsi transférés dans les réseaux trophiques avec une augmentation des concentrations entre ces niveaux (biomagnification).

III. LA BIOACCUMULATION DES METAUX

III.1. DEFINITIONS

III.1.1. Biodisponibilité

La *biodisponibilité* représente la quantité de contaminants disponible pour l'assimilation par les organismes (Newman & Jagoe 1994). Certains considèrent même que la fraction biodisponible des métaux est la seule qui doit être susceptible d'induire un effet sur l'organisme (Campbell 1995). La biodisponibilité dépend de l'espèce, du métal, et de sa spéciation dans l'environnement ambiant. La spéciation des métaux est considérée comme un des premiers facteurs limitant la biodisponibilité des métaux, et agit donc sur l'assimilation par les organismes (Nelson & Donkin 1985). A titre d'exemple, la biodisponibilité du mercure élémentaire (Hg^0) est accrue dans les sédiments riches en matières organiques et réducteurs, car ce dernier sera converti par des bactéries anaérobies en méthyl-mercure (Jensen & Jernelov 1969), plus facilement assimilable pour les organismes (Wolfe et al. 1998).

Campbell (1995) considère que la toxicité d'un métal est mieux prédite par la concentration en ions métalliques libres et par certains complexes, que par la concentration totale en métaux. Ainsi, dans le cas du Cd, la toxicité s'avère plus liée à sa forme ionique plutôt qu'au métal total (Sunda et al. 1978; Sanders & Cibik 1985). Ces résultats montrent alors tout l'intérêt en toxicologie aquatique, de prendre en compte la fraction biodisponible des métaux et non pas la concentration métallique totale.

III.1.2. Bioaccumulation, Bioconcentration et Bioamplification

La *bioaccumulation* désigne la capacité des organismes de concentrer dans leurs tissus un contaminant par voie directe et/ou alimentaire, même si ce dernier n'a aucun rôle métabolique et même s'il est toxique pour l'organisme. L'ECETOC (1996) (European Chemical Industry Ecology and Toxicology Center) définit la bioaccumulation comme « *le résultat net de l'accumulation, la distribution et l'élimination d'une substance dans un organisme due à son exposition dans l'eau, la nourriture, les sédiments et l'air* ». D'un point de vue écotoxicologique, la bioaccumulation résulte du bilan entre les entrées et les différentes voies d'élimination des métaux dans les organismes. L'entrée des métaux dans les organismes résulte de processus d'adsorption et d'absorption au travers des barrières biologiques.

La *bioconcentration* est un cas particulier de la bioaccumulation. Elle désigne l'accroissement direct de la concentration d'un contaminant lorsqu'il passe de l'eau à un organisme aquatique. L'accroissement de la concentration d'un contaminant entre un organisme aquatique et son environnement peut-être représenté par le facteur de concentration (FC), rapport de la concentration d'un contaminant dans un organisme à sa concentration dans l'eau de mer.

La *bioamplification* désigne l'augmentation progressive de la concentration d'un contaminant le long du réseau trophique, à l'intérieur des biocénoses contaminées. Plus précisément, la bioamplification se définit comme le transfert d'un contaminant de la nourriture à un organisme, avec au final une concentration plus forte dans l'organisme que dans la nourriture (Connell 1990; Rand et al. 1995; Connell et al. 1998). La bioamplification n'est pas un processus fortement répandu et seuls certains métaux sont bioamplifiés le long des chaînes trophiques (e.g., le Hg sous sa forme méthylée, Riisgaard & Hansen 1990; Francesconi & Lenanton 1992; le Se, Barwick & Maher 2003).

Ainsi, le devenir d'un contaminant dans un biotope contaminé peut se résumer selon trois cas : soit une diminution de la concentration du contaminant le long du réseau trophique; soit un simple transfert du contaminant; soit une amplification biologique dans les échelons supérieurs des chaînes trophiques. Ces différentes possibilités ont été clairement illustrées par Ramade (1992) repris ici par la figure 3, qui montre les principaux types de pyramides des

concentrations qu'il est possible d'observer en fonction de la valeur du facteur de transfert dans les réseaux trophiques.

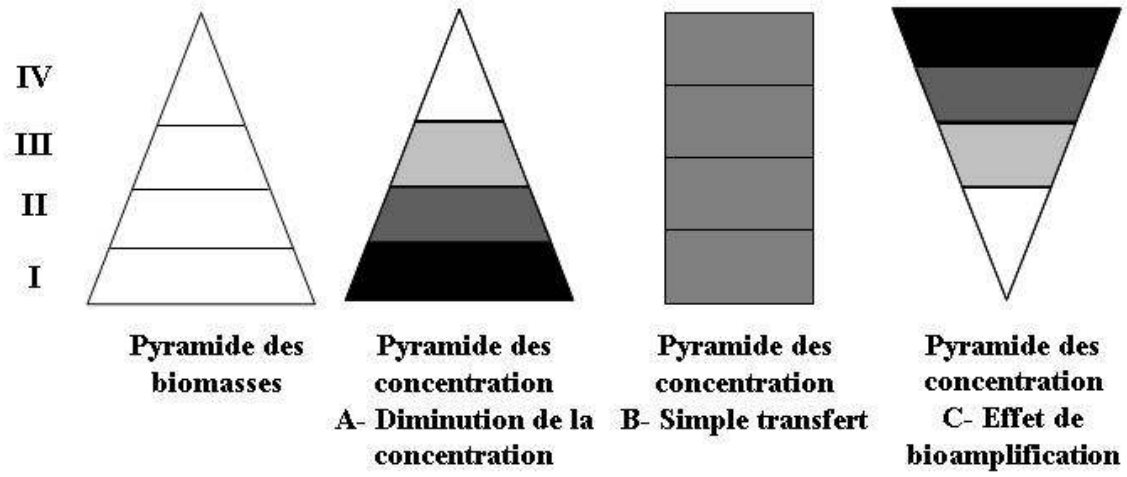

Figure 3. Principaux types de pyramides des concentrations selon la valeur du facteur de transfert dans les réseaux trophiques (Source : Ramade 1992).

III.2. FACTEURS INFLUENÇANT LA BIOACCUMULATION

La bioaccumulation des métaux dans les organismes peut-être divisée en plusieurs phases : (1) la phase d'accumulation des métaux, (2) la phase de transport, distribution et séquestration des métaux, et enfin (3) la phase d'excrétion des métaux. Les relations entrent ces différents processus définissent les stratégies d'accumulation des organismes (Phillips & Rainbow 1989; Rainbow 1993b). La bioaccumulation n'est pas un phénomène uniforme. Les mécanismes de bioaccumulation des éléments métalliques dans les organismes aquatiques varient en fonction de deux types de facteurs : (1) les facteurs biotiques caractérisant les paramètres biologiques et physiologiques des êtres vivants (espèce, âge, période du cycle reproducteur, aptitude à métaboliser certains métaux, régime alimentaire, pré-exposition aux métaux); (2) les facteurs abiotiques regroupant les paramètres physico-chimiques de l'écosystème qui peuvent eux-mêmes être interdépendants (e.g., spéciation des métaux, température, salinité, pH, potentiel redox), et les conditions d'exposition aux contaminants (concentration métallique, caractéristiques des voies d'accumulation) (Laws 1993). En conditions naturelles, ces différents facteurs sont caractérisés par une très grande diversité et par des variations spatiales et temporelles. L'influence des différents facteurs écologiques, physiologiques, physico-

chimiques sur l'accumulation des métaux dans les organismes doit être prise en compte avant de mesurer et de comparer les concentrations métalliques dans les tissus des organismes sélectionnées comme bioindicateurs (Phillips 1980; Cossa 1989).

III.2.1. Facteurs biotiques

III.2.1.1. Influence de l'espèce

L'accumulation des métaux dans les organismes est fonction de l'espèce considérée (e.g., Eisler 1981; Miramand et al. 1999). Les organismes marins appartenant à un même écosystème et soumis aux mêmes concentrations métalliques dissoutes et particulaires montrent des capacités de bioaccumulation différentes (Miramand et al. 1999). De même, des organismes marins vivant dans le même habitat peuvent avoir des concentrations métalliques très variées (Phillips & Rainbow 1993). Des différences de concentration existent au sein d'une même famille et/ou du même genre (Rainbow 1993b). Dans des eaux polluées, alors que les bivalves accumulent de grandes quantités de métaux dans leurs tissus, les crustacés peuvent mettre en place un système de régulation assurant ainsi le contrôle de leurs concentrations métalliques (Phillips & Rainbow 1993). Des organismes capables de réguler leurs concentrations pour certains métaux ne présentent aucun intérêt en tant que bioindicateurs. En effet, ils ne seront pas capables de refléter les variations des concentrations métalliques ambiantes. Les bivalves sont généralement choisis comme bioindicateur de la contamination métallique car ils présentent peu de capacité de régulation. Cependant les capacités d'accumulation des bivalves sont sous influence physiologique. Par exemple, le comportement accumulateur de l'Ag dans trois bivalves montre un pouvoir de bioconcentration pour les huîtres plus important que pour les coquilles Saint Jacques ou les moules (Metayer et al. 1990). Une autre étude montre l'inégalité des capacités de concentration des moules et des huîtres, notamment vis-à-vis du zinc. Les concentrations en Zn détectées dans les huîtres *Crassostrea virginica, Saccostrea cucullata* et *Ostrea edulis* sont 1 à 3 ordres de grandeur supérieurs à celle mesurées dans les moules *Mytilus edulis, Perna viridis* et *Septifer virgatus* (Rainbow 1993a). Les huîtres sont capable d'accumuler de fortes concentrations de Zn sous forme de granules (George et al. 1978) alors que les moules excrètent fortement le Zn accumulé dans les granules des reins (George & Pirie 1980).). Les huîtres sont ainsi considérées comme de puissants accumulateurs de Zn (Phillips & Yim 1981) et les moules comme de faibles accumulateurs voire régulateurs de Zn. Néanmoins, les différences d'accumulations entre deux espèces n'existent pas toujours, et les moules *M.*

edulis et *M. californianus* collectées à la même période dans la rivière Columbia ne montrent pas de différence d'accumulation (O'Connor 1993). Ainsi, chaque organisme possède des capacités de bioaccumulation qui lui sont propres, ces dernières étant susceptibles d'induire des répercussions au niveau de l'utilisation des espèces comme bioindicateurs.

III.2.1.2. Influence de la taille (âge) des organismes

Les concentrations métalliques dans les organismes sont corrélées avec la taille des organismes (Boyden 1974, 1977). La taille est généralement reliée à l'âge des organismes, cependant ce n'est pas toujours pas le cas. Les relations allométriques varient en fonction des espèces et des métaux. En général, la croissance exerce un effet de dilution de la concentration métallique (Boyden 1974). Deux hypothèses expliquent ce phénomène : (1) une diminution du rapport surface/volume avec l'âge, (2) une diminution de l'activité métabolique chez les individus les plus âgés. La bioaccumulation chez les jeunes individus est plus rapide due à une plus grande efficacité d'absorption, et à une demande croissante en énergie nécessaire à la croissance (Fagerstromn 1977; Lobel et al. 1982). Les études menées en laboratoire (Fowler & Benayoun 1974) ou en milieu naturel (Cossa 1980) confirment cette capacité d'accumulation supérieure chez les jeunes individus.

Néanmoins, d'après les différents travaux traitant du sujet, il n'existe pas de tendance unique. Dans certains cas, les concentrations métalliques les plus fortes sont mesurées chez les individus les plus âgés. Strong & Luoma (1981) ont observé une corrélation positive entre les concentrations d'Ag dans les tissus mous et la taille du clam *Macoma balthica*. Dans d'autres cas, les concentrations métalliques restent inchangées sur toute une gamme de taille (e.g., le Zn dans la moule bleu *Mytilus edulis*, Williamson 1980; Brix & Lyngby 1985)

La taille des organismes influence non seulement les concentrations métalliques dans les tissus, mais elle influence aussi certains paramètres physiologiques comme la consommation d'oxygène ou le taux de filtration (e.g. Widdows 1978; Hamburger et al. 1983). La variation des paramètres physiologiques reliés au métabolisme de l'organisme et aux besoins énergétiques de l'organisme à un âge donné peut permettre de comprendre les variations de concentrations observées.

III.2.1.3. Influence du sexe et du cycle sexuel

De nombreuses études se sont intéressés à l'influence du sexe de l'organisme sur les concentrations métalliques (e.g., Cossa et al. 1979; Lobel & Wright 1982). Les mesures de

concentrations métalliques plus fortes chez les mâles que chez les femelles de *Mytilus edulis* (La Touche & Mix 1982), et *Choromytilus meridionalis* (Watling & Watling 1976; Orren et al. 1980) pourraient s'expliquer par les nombreux lysosomes contenant des granules de zinc dans les reins des *Mytilus edulis* mâles (Lowe & Moore 1979). Néanmoins ces différences ne sont pas toujours significatives (e.g., la moule *Perna viridis*, Phillips 1985).

Le cycle sexuel induit des changements physiologiques dans les organismes, capable d'influencer les concentrations métalliques des organismes. En effet, la maturation des gonades s'avère un facteur important dans la fluctuation du poids des tissus, exerçant généralement un effet de dilution (La Touche & Mix 1982). D'autre part, des variations de concentrations en Cd plus élevées chez les adultes que chez les jeunes individus de *Mytilus edulis* démontrent l'influence de la maturation sexuelle sur le potentiel bioaccumulateur , probablement dû à des changements biochimiques liés à leurs cycles sexuels (Cossa et al. 1979). Le développement gonadique étant difficilement prévisible, il est dès lors déconseillé d'utiliser des moules sexuellement matures pour suivre les concentrations métalliques. L'état du développement reproductif est un facteur à prendre en compte dans le suivi des concentrations métalliques des organismes lors d'un programme de biosurveillance (Cossa et al. 1979).

III.2.1.4. Pré-exposition aux métaux

La pré-exposition (naturelle ou en laboratoire) des organismes aux contaminants métalliques peut provoquer une modification du comportement bioaccumulateur (e.g., Wang & Rainbow 2005) et quelque fois, une adaptation génétique (Klerks & Weis 1987). Un des premiers travaux étudiant l'adaptation des plantes aux métaux toxiques est celle de Gregory & Bradshaw (1965). Par la suite, d'autres travaux ont également mis en évidence des phénomènes d'adaptation aux métaux toxiques pour les organismes aquatiques. Par exemple, Bryan a comparé la CL_{50} de populations d'Annélides polychètes (*Nereis diversicolor*) provenant de divers estuaires d'Angleterre, dont les sédiments étaient pollués par des mines de Cu et d'autres métaux, à celle de souches de *N. diversicolor* provenant de biotopes témoins. La CL_{50} des populations vivants dans des endroits non pollués s'avère cinq fois plus faible (CL_{50} = 0,5 µg cm^{-3}) que celle vivant dans des endroits pollués (CL_{50} = 2,5 µg cm^{-3}) (Bryan 1976; Bryan et al. 1985). Ceci démontre la tolérance des Annélides vivant sur des sédiments riches en Cu. Les organismes adaptés aux conditions environnementales sont plus à même de tolérer l'exposition aux contaminants, que ne le feraient la même espèce vivant dans

un milieu non contaminé. Les organismes vivants dans des régions fortement contaminées peuvent donc se révéler moins sensibles aux métaux que les organismes vivants dans des milieux faiblement contaminés.

Afin d'approfondir l'influence de la pré-exposition aux métaux sur les organismes, plusieurs études ont pré-exposé en laboratoire des organismes à différents métaux, avant de les exposer aux métaux concernés. La pré-exposition à un métal particulier peut avoir une influence sur l'assimilation de ce dernier. Par exemple, il a récemment été démontré l'influence positive de la pré-exposition de la moule *Perna viridis* au Cd sur l'assimilation de ce dernier (Blackmore & Wang 2002; Shi & Wang 2004). Mais la pré-exposition à un métal peut aussi avoir une influence sur le comportement d'autres métaux. La pré-exposition de la moule *Mytilus edulis* au Cd ou Cu provoque une augmentation de la tolérance au Hg (II). En effet, le Hg (II) présent va pouvoir se lier aux métallothionéines présentes, dont la synthèse a été induite lors de la pré-exposition au Cd ou au Cu. Le Hg (II), ainsi stocké est moins toxique pour l'organisme (Roesijadi & Felligham 1987).

Dans un contexte de surveillance des concentrations métalliques dans les organismes, ces modifications du comportement bioaccumulateur suite à une pré-exposition aux métaux vont à l'encontre du critère principal d'un bioindicateur. En effet, un bioindicateur provenant de différentes localisations doit répondre de la même manière aux métaux présents dans un site donné. Les différences observés dans les concentrations métalliques des bioindicateurs doivent être attribuables uniquement à des différences de biodisponibilités des métaux dans les sites (e.g., Phillips & Rainbow 1993). Ainsi, récemment l'intérêt s'est porté sur l'étude des capacités d'accumulation d'organismes ayant des histoires de contaminations différentes. Rainbow et al. (1999) a mesuré les taux d'accumulation de métaux dissouts dans des crabes (*Carcinus maenas* et *Pachygrapsus marmoratus*) et des crustacés (*Orchestia gammarellus*) provenant de sites côtiers enrichis différemment en métaux. Les taux d'accumulation de l'Ag, Cd et du Zn d'organismes provenant de sites contrôles et de sites enrichis en métaux ne présentent pas de différence importante. La pré-exposition historique aux métaux des organismes n'a pas d'effet significatif sur le taux d'accumulation de l'Ag, Cd et Zn. Ce résultat a été confirmé pour le Cd et le Zn (Rainbow 1993b; Rainbow et al. 2004). Dès lors, l'utilisation de bioindicateurs dans des programmes de biosurveillance ne serait pas compromise par la pré-exposition naturelle des organismes vivants dans les différents sites.

III.2.1.5. Interaction des métaux

Entre eux, les métaux peuvent avoir des effets antagonistes, synergiques ou nuls sur la santé des animaux (e.g., Strömgren 1986; Widdows & Donkin 1992). Les effets antagonistes se traduisent généralement par l'existence d'une compétition entre les métaux pour certains sites de liaisons ou d'accumulation. Par exemple, la présence de fortes concentrations de Cd, Cu ou Mn en solution diminue fortement le taux d'accumulation du ^{65}Zn dans l'algue brune *Laminaria digitata*, impliquant l'existence d'une compétition du Cd et du Mn par rapport au Zn (Bryan 1969).

Au contraire, certains métaux ont des effets synergiques: des poissons exposés à un mélange de Cu et de Cd ont une capacité de concentration plus importante, que lorsque ces mêmes poissons sont exposés aux deux métaux séparément (Hewitt & Anderson 1978). Le Zn et le Cd, en raison de leurs propriétés physico-chimiques similaires, entrent en compétition pour les mêmes sites de liaisons dans les métallothionéines (Wittman 1979).

III.2.1.6. Organotropisme

Les métaux ne sont pas répartis de manière uniforme dans les organismes. Certains organes (e.g. reins, glande digestive) ont des capacités de concentration vis-à-vis des métaux plus importantes que d'autres organes (e.g. muscle, pied). George & Coombs (1977) ont établit un classement pour les concentrations en Cd dans la moule *Mytilus edulis*: reins > viscères > branchies > manteau > muscle, confirmé par la suite par Everaarts (1990). Néanmoins, selon les espèces et les métaux, ce classement peut être différent. Par exemple, dans la moule *Perna viridis*, le Hg, le Cu, le Zn et le Pb sont accumulés dans les organes dans l'ordre décroissant : branchies > viscères > muscle > manteau (Lakshmanan & Nambisan 1989).

III.2.2. Facteurs abiotiques

III.2.2.1. Effet de la saison, température et salinité

La saison, la température et la salinité sont des facteurs écologiques abiotiques connus pour influencer la bioaccumulation des contaminants. De nombreux auteurs ont reporté des variations significatives des concentrations métalliques en fonction de la saison dans les algues (e.g., Fuge & James 1973; Burdon-Jones et al. 1982; Miramand & Bentley 1992) ou dans les bivalves (e.g., Boyden & Phillips 1981; Shulkin & Kavun 1995). Les variations saisonnières des concentrations métalliques s'expliquent par des variations associées à la nourriture (Bryan 1973), aux changements de l'activité physiologique de l'organisme (e.g.,

taux de filtration, cycle reproducteur) (Riedel et al. 1998), à l'activité biologique et biochimique (activité microbienne, assimilation de phytoplancton) et aux changements de la biodisponibilité des métaux (Paez-Osuna et al. 1993). Dans le cas des algues, les variations de concentrations métalliques sont généralement associées aux changements saisonniers de la croissance des algues. Les concentrations métalliques maximales sont mesurées au printemps et les concentrations minimales en automne (e.g., Fuge & James 1974; Young 1975). Les forts taux de croissance observés pendant la saison chaude de l'année entraînent un effet de dilution des métaux accumulés.

Les variations saisonnières sont intimement associées à des changements de température et de salinité, pouvant influencer les concentrations métalliques dans les organismes. Concernant la salinité, de nombreux travaux montrent l'existence d'une relation inverse entre la salinité et l'accumulation des métaux, en laboratoire ou sur le terrain (e.g., Phillips 1976a, 1977a; Fisher 1986; Miramand et al. 1998). Ainsi, en hiver, la diminution de la salinité est corrélée avec une augmentation du taux d'accumulation des métaux. Dans le cas du Cd, l'accumulation par l'algue brune *Fucus vesiculosus* est favorisée aux faibles salinités (Bryan et al. 1985). En effet, la salinité est connue pour influencer la spéciation chimique des métaux (Sunda et al. 1978; Wright 1995), et par la même, la biodisponibilité des métaux dans l'environnement et l'accumulation des métaux par les organismes (Phillips 1976a).

Les variations de température sont aussi souvent corrélées avec des variations de concentrations métalliques. Les taux d'accumulation et d'élimination du ^{65}Zn dans l'algue *Ulva lactuca* et *Porphyra umbilicalis* sont plus rapides aux fortes températures (Gutknecht 1961, 1963). De même l'algue *Fucus vesiculosus* présente des facteurs de concentrations dépendant de la température pour certains éléments (^{54}Mn, ^{60}Co, ^{65}Zn, ^{109}Cd, ^{110m}Ag) avec une diminution significative aux faibles températures (2°C, Boisson et al. 1997). Cependant, une baisse de la température diminue le taux d'accumulation des métaux (Fisher 1986) mais ne provoque pas forcément de variation du taux d'élimination (Fisher 1986; Hutchins et al. 1996).

III.2.2.2. La spéciation chimique

La forme chimique du métal a une importance capitale sur sa biodisponibilité pour les organismes. En effet, la spéciation est un des premiers facteurs modifiant et limitant l'accumulation des métaux par les organismes (Nelson & Donkin 1985). Par exemple, dans les eaux naturelles, le Cr existe à l'état d'oxydation III et VI, ce dernier étant fortement

toxique pour les organismes et l'écosystème. Des expériences ont montré que le Cr (VI) était accumulé trois fois plus vite sous forme dissoute et également mieux assimilé via la nourriture que le Cr (III) (e.g., Wang et al. 1997). La spéciation conditionne donc l'accessibilité des métaux aux barrières biologiques et leur transport vers les compartiments internes. Cependant la spéciation des métaux dans l'eau de mer est très difficile à déterminer, notamment à cause de la matrice très complexe de l'eau de mer.

III.2.2.3. Caractéristiques des sédiments

Le compartiment sédimentaire peut influencer la bioaccumulation des métaux présents dans l'environnement. En effet, des corrélations entre les concentrations métalliques dans les organismes marins et celle des sédiments ont été observées (O'Connor & Ehler 1991; Ringwood et al. 1999; Soto-Jimenez et al. 2001). Parmi les bivalves, les organismes fouisseurs ou bivalves déposivores sont davantage sujets à une contamination via les sédiments de part leur régime alimentaire. D'autres part, une étude sur l'absorption du ^{65}Zn, ^{60}Co et ^{110m}Ag par la telline de la Baltique *Macoma balthica* à partir de différents sédiments montrent que la disponibilité des métaux est dépendante du type de sédiment et inversement corrélée à la force de la liaison métal-sédiment. La biodisponibilité des métaux liés aux particules de sédiment est fortement dépendante du type de particules (Bryan 1980; Mountouris et al. 2002). Le contenu organique des sédiments (e.g., Harvey & Luoma 1985; Mahony et al. 1996; Gagnon & Fisher 1997) et/ou l'enrichissement en hydroxydes de fer et de Mn des sédiments (Bendell-Young & Harvey 1991; Pempkowiak et al. 1999) rendent les métaux associés plus biodisponibles pour les organismes. Les métaux adsorbés aux hydroxydes de Fe ou de Mn, aux carbonates, à la matière organique et aux particules d'argile sont plus facilement échangeables et donc plus biodisponibles pour les organismes (Salomons et al. 1987). Cependant, du fait de la grande variété des types de sédiments dans l'environnement, les liaisons des métaux aux particules sédimentaires sont diverses et variées; et la biodisponibilité des métaux liés aux particules de sédiments est relativement difficile à prévoir (Luoma 1989).

III.2.2.4. Caractéristiques du phytoplancton

Les organismes marins, et particulièrement les bivalves sont capables de s'adapter aux variations des conditions de nourriture présente dans l'environnement (Widdows & Donkin 1992). En effet, ils disposent de mécanismes compensatoires, leur permettant de réguler la capture de la matière particulaire et d'optimiser l'énergie ingérée (Bayne 1993; Navarro &

Iglesias 1993). Ils possèdent des mécanismes efficaces de sélection de la matière particulaire : ils peuvent rejeter comme pseudofèces les particules pauvres au niveau nutritif (matières inorganiques), et retenir les particules fortement nutritives (matières organiques) (Jorgensen 1996; Arifin & Bendell-Young 1997). Ainsi des paramètres comme la qualité du seston (Velasco & Navarro 2002), la concentration de la nourriture (Wilson 1983b; Iglesias et al. 1992; Wang et al. 1995), et la taille des particules (Mohlenberg & Riisgard 1978; Jorgensen 1996) influencent l'efficacité de rétention et l'assimilation de la nourriture et des métaux associés aux cellules phytoplanctoniques. Par exemple, le Cd associé à la diatomée *Skeletonema costatum* est plus faiblement retenu par l'huître *Crassostrea gigas* (9%, après 21 jours d'exposition) que celui associé à la prasinophyte *Tetraselmis suecica* (20%) (Ettajani et al. 2001). La différence de répartition des métaux dans le cytoplasme des algues peut-être une explication, la fraction cytoplasmique étant la plus biodisponible pour les bivalves (Lee & Luoma 1998).

III.2.2.5. La concentration métallique

Les organismes sont susceptibles d'être exposés à des concentrations métalliques très contrastées (> 2 ordres de grandeurs). Pour être informative, une espèce bioindicatrice doit renseigner sur l'état de contamination du milieu dans lequel elle vit. Et pour cela, elle doit nécessairement accumuler les métaux de façon proportionnelle à la concentration en ces métaux présents dans le milieu ambiant (Bryan et al. 1980). Cependant, à partir d'une certaine concentration, une modification du comportement dans les organismes peut avoir lieu avec la mise en place d'un système de régulation (Bryan 1976). Par exemple, la moule *Mytilus galloprovincialis* exposé à une gamme de concentrations d'As (de 1 à 100 $\mu g\ l^{-1}$), pendant 13 jours, montre une diminution du taux d'accumulation aux plus fortes concentrations (Ünlü & Fowler 1979). Il apparaît donc primordial de caractériser le comportement bioaccumulateur des organismes en fonction de l'intensité de la contamination ambiante. Bien qu'il s'agisse probablement là ***du principal critère*** que doit nécessairement présenter un organisme pour pouvoir être considéré et utilisé comme bioindicateur, très peu d'études se sont attardées à le vérifier effectivement (e.g., Miramand et al. 1980; Zaroogian 1980; Talbot 1985; Bjerregaard 1988; Warnau et al. 1997). Le Cd est un des métaux pour lesquels le plus d'études portant sur ce critère existe. La cinétique du ^{109}Cd chez *Mytilus galloprovincialis*, étudié par Fowler & Benayoun (1974), montre un taux d'absorption du Cd directement proportionnel à sa concentration dans l'eau de mer (0,1-100 $\mu g\ l^{-1}$). Ce résultat est confirmé par d'autres travaux sur le Cd (e.g., George & Coombs 1977; Scholz 1980), et sur le Ni chez la moule *Mytilus*

edulis exposé à une gamme allant de 18 à 107 μg l^{-1} (Friedrich & Fillice 1976). Ceci démontre la capacité des moules *Mytilus* spp. à accumuler les métaux dans des environnements pollués à différents degrés. Néanmoins, à part ses quelques études éparses, la tendance est plutôt de considérer que les espèces utilisées comme indicateurs se conforment *de facto* à ce pré requis. Selon les spécialistes, ce choix relève très généralement d'une solution de facilité plus que d'un comportement scientifique En effet, généralement la sélection des bioindicateurs dépend plus de la disponibilité des espèces dans les zones étudiées que de leur réelle capacité à refléter la contamination ambiante (e.g., Phillips 1990; Rainbow & Phillips 1993; Warnau 1996).

III.2.2.6. La voie d'exposition

Dans l'environnement marin, les métaux peuvent être présent sous différentes formes (dissoutes ou particulaire). Les organismes marins sont donc exposés aux métaux via trois sources d'accumulation majoritaires (eau de mer, sédiments, phytoplancton) et les capacités d'accumulations des organismes sont différentes selon chaque source. L'importance de chaque source dans la bioaccumulation totale du métal dans l'organisme dépend de la concentration du métal dans chacune des voies et des aptitudes biologiques de l'organisme (e.g. taux d'ingestion, taux de filtration). De nombreuses expériences se sont intéressées à déterminer quelle était la voie majoritaire d'accumulation des métaux dans les organismes. Borchardt (1983; 1985) a voulu déterminer quel était le vecteur préférentiel d'absorption du Cd entre l'eau et la nourriture. Il a utilisé du ^{115m}Cd pour marquer l'eau et du ^{109}Cd pour la nourriture. L'absorption du Cd par voie dissoute s'est avérée prédominante chez la moule *Mytilus edulis*. Au contraire d'autres études sur l'accumulation du Cd, Mn et Zn ont montré l'importance de la voie particulaire (phytoplancton et autres matières particulaires) (Fowler & Benayoun 1974) dans différents bivalves tels que les huîtres, les coquilles Saint-Jacques (Bryan 1973) ou les clams déposivores (Bryan & Uysal 1978). Cependant, la détermination de la voie d'accumulation majoritaire dans les organismes était souvent basé sur la comparaison des facteurs de concentration ou des vitesses d'accumulation des métaux (e.g., Riisgard et al. 1987; Metayer et al. 1990; Scanes 1993; Fisher et al. 1996). Or, il est important de prendre en compte la concentration métallique de chaque compartiment. Les métaux sont plus concentrés dans les sédiments (Constante de distribution -K_d- supérieur à 10^2) et dans le phytoplancton que dans l'eau de mer. Ainsi, une accumulation plus rapide des métaux dissouts ne signifie pas forcément une prédominance de la voie dissoute (Preston 1971). Afin de palier à ce manque d'intégration des données, et d'estimer la contribution relative de

chacune des voies à la contamination globale de l'organisme, un modèle de bioaccumulation a été développé par Thomann (1981) et Landrum et al. (1992) ; et amélioré par la suite (e.g., Wang et al. 1996; Reinfelder et al. 1998).

III.2.2.7. La symbiose des animaux

Les organismes symbiotiques peuvent influencer la bioaccumulation de certains organismes. Les zooxanthelles ont cette capacité. Par exemple, des concentrations très élevées en arsenic ont été détectées dans le clam géant *Tridacna maxima* et *T. derasa* de la Grande Barrière, atteignant 1000 $\mu g\ g^{-1}$ dans les reins (Benson & Summons 1981) alors que l'arsenic est généralement faiblement accumulé dans les organismes. Une telle concentration peut s'expliquer par l'intervention des zooxanthelles symbiotiques du manteau du clam. En effet, ces dernières accumulent l'arsenic à partir de l'eau et le convertissent sous forme organique (Phillips 1994a) avant de le transférer à leur ôte. Les zooxanthelles sont présentes dans d'autres organismes, comme les anémones de mer ou les coraux. Des études sur les zooxanthelles symbiotiques des anémones et des coraux ont mis en avant leur rôle dans l'accumulation (Harland et al. 1990; Harland & Nganro 1990) et dans la régulation des métaux (Harland & Brown 1989; Reichelt-Brushett & McOrist 2003).

IV. LE CONCEPT DES BIOINDICATEURS

Selon le principe de prévention et précaution, énoncé pour la première fois lors de la conférence internationale sur la protection de la mer du Nord en 1987, il est apparu primordial de pouvoir préserver le milieu marin des pollutions. Cependant, les difficultés liées à la préservation du milieu marin se sont clairement fait ressentir par la suite. La mer n'appartient à personne et les pollutions dans le milieu marin ne connaissent pas de frontière. Aujourd'hui, si des accords entre les états ont été adoptés sur la protection de la mer et de ses ressources, c'est bien souvent parce que l'environnement est considéré comme un enjeu économique important (Pearce & Moran 1994). En effet, les impacts d'une pollution constituent bien souvent une perte économique liée à une diminution de la pêche, des valeurs des produits de la mer et du tourisme. Inversement, la classification d'un site au patrimoine mondial de l'U.N.E.S.C.O (United Nations Educational Scientific and Cultural Organization) constitue un élan pour l'activité touristique. Cette notion de mise en valeur des ressources est assez récente Et Edward Wilson écrivait en 1992 : « *la biodiversité est l'une des plus grandes richesses de la planète, et pourtant la moins reconnue comme telle* ».

Afin de surveiller la présence excessive de métaux lourds dans l'environnement marin, constituant dès lors une menace pour les organismes et les écosystèmes, il est essentiel de disposer d'outils qui permettent d'évaluer rapidement et de manière fiable l'état de la contamination métallique de l'environnement. La surveillance des contaminants dans l'environnement peut se faire selon deux approches, qui fournissent des informations complémentaires : (1) la détection des contaminants et leur quantification dans les différents compartiments de l'écosystème considéré; (2) l'évaluation de leurs effets biologiques à différents niveaux d'organisation du monde vivant (de la molécule à l'écosystème entier). De façon optimale, ces deux approches devraient être menées en parallèle. Cependant par rapport aux besoins déterminés, la première approche a été choisie pour évaluer le degré de contamination du milieu. Cette quantification est possible dans différents compartiments de l'écosystème : soit sur le compartiment physique en analysant les métaux présents dans le compartiment sédimentaire et/ou dans l'eau de mer; soit dans le compartiment biologique, en analysant les métaux présent dans les organismes marins (animaux ou végétaux).

IV.1. MESURES DANS LES DIFFERENTS COMPARTIMENTS

IV.1.1. Compartiments physiques

Suivre la contamination métallique dans l'eau de mer apparaît difficile à bien des niveaux. Tout d'abord au niveau analytique, les métaux dissouts dans l'eau de mer sont présents à l'état de trace avec des concentrations extrêmement faibles, i.e. de l'ordre du ng l^{-1} (Tab. 3) ; leur analyse nécessite donc des moyens techniques extrêmement sensibles et coûteux. De plus, les procédures d'analyses comportent toujours le risque d'une contamination accidentelle lors de la préparation des échantillons. Aux inconvénients analytiques s'ajoutent des problèmes de représentativité. Les masses d'eau ne sont pas homogènes et une forte variabilité des concentrations métalliques est associée à la position et à la profondeur des prélèvements d'eau de mer. Enfin, la mesure des concentrations métalliques dans l'eau de mer, sans prise en compte de leur spéciation, ne permet pas de prévoir la biodisponibilité des métaux pour les biocénoses (Brezonik et al. 1991).

Éléments	Concentration
Ag	0,05 - 3,77 ng l^{-1}
As	1,124 - 1,873 µg l^{-1}
Cd	0,11 - 123.6 ng l^{-1}
Co	0,6 - 5,9 ng l^{-1}
Cr	104 - 260 ng l^{-1}
Cu	31,8 – 381,8 ng l^{-1}
Fe	5,6 - 139,6 ng l^{-1}
Hg	0,4 - 2 ng l^{-1}
Mn	11 - 164,8 ng l^{-1}
Ni	0,117 - 0,704 µg l^{-1}
Pb	1,04 - 36,26 ng l^{-1}
Zn	3,3 - 588,5 ng l^{-1}

Tableau 3. Gamme de concentration des éléments dissouts dans l'eau de mer à une salinité de 35 p.s.u. (Source : Bruland 1983).

Les métaux parviennent à l'océan sous différentes formes (e.g. dissoute, particulaire) (Goldberg 1954). Nombre d'entre eux vont ensuite s'adsorber à des matières particulaires en suspension et sédimenter au bout d'un certain temps (Salomons & Förstner 1984). Les sédiments sont donc souvent considérés comme le réceptacle ultime des contaminants qui arrivent dans l'Océan, et les concentrations métalliques dans les sédiments sont de plusieurs ordres de grandeur supérieurs à celles mesurées dans l'eau de mer (Bryan & Langston 1992). Un des avantages de l'analyse des métaux dans les sédiments réside dans la facilité d'analyse et la réduction du coût par rapport aux analyses métalliques dans l'eau de mer. Cependant, plusieurs inconvénients existent dans l'interprétation des données concernant les concentrations métalliques dans les sédiments. Tout d'abord, la notion de temps. La concentration d'un métal dans les sédiments n'est pas seulement une fonction de la quantité de métal déposé, mais une fonction du rapport de la quantité déposée sur les sédiments sur une période de temps donné. L'analyse des sédiments donne donc une information intégrée dans le temps. Ensuite, la concentration des métaux dans les sédiments dépend de leur contenu en matière organique. Et enfin, les mesures des métaux dans les sédiments ne donnent pas d'information sur la disponibilité des métaux pour les biocénoses (Nelson & Donkin 1985). Selon certains auteurs, ce dernier point est discutable. Les techniques

d'extraction séquentielle (Perin et al. 1997; Akcay et al. 2003) fournissent des informations sur l'origine, la mobilité et la disponibilité des éléments liés aux différentes phases géochimiques des sédiments (échangeable, lié aux carbonates, lié aux oxydes de Fe et Mn, liés à la matière organique et à la phase résiduelle) et renseignent donc sur la fraction métallique biodisponible des sédiments (e.g., Tessier et al. 1979; Tessier & Campbell 1987; Ankley et al. 1996).

Cependant, la fraction métallique biodisponible des sédiments ne représente pas forcément la fraction réellement biodisponible pour les organismes (Arjonilla et al. 1994). Ainsi, la manière la plus directe de quantifier la fraction biodisponible pour les organismes consiste à mesurer la bioaccumulation des éléments métalliques par les organismes (Borgmann et al. 2001).

IV.1.2. Compartiments biologiques

Pour évaluer le degré de contamination des zones côtières, l'utilisation d'organismes marins comme indicateurs de la contamination d'une zone littorale apparaît comme une alternative introduisant des valeurs biologiques par rapport aux mesures des concentrations métalliques dans l'eau ou les sédiments. La capacité des organismes marins de bioaccumuler les métaux présents dans l'environnement, de plusieurs dizaines à plusieurs milliers de fois au-delà de la concentration existant dans le milieu, permet d'utiliser ces organismes comme « indicateur de contamination » ou bioindicateurs. L'utilisation de bioindicateurs présente de multiples avantages tant au niveau analytique qu'au niveau de l'information obtenue. Les analyses à partir d'échantillons d'organismes marins sont facilitées par rapport aux échantillons d'eau, de part les capacités de bioaccumulation des organismes. De plus, les processus d'accumulation et de dépuration de l'organisme prennent un certain temps, ce qui élimine l'influence des variations instantanées et permet une préservation de l'information de la contamination sur de longues périodes (quelques semaines à plusieurs mois). Et enfin la mesure des concentrations métalliques dans les organismes permet une mesure directe de la biodisponibilité des métaux présents dans l'environnement (Phillips 1980; Waldichuk 1985).

L'estimation de la fraction dite « biodisponible » représente une information très importante puisqu'elle est à l'origine des éventuels phénomènes de toxicité.

IV.2. NOTION DE BIOINDICATEUR

Le terme « bioindicateur » regroupe de nombreuses définitions. En effet, les effets biologiques d'une contamination métallique peuvent apparaître à différents niveaux d'organisation : au niveau infra-individuel, individuel, de la population, des communautés et de l'écosystème. En fonction du niveau d'organisation, différents bioindicateurs seront distingués : les bioindicateurs écologiques, les biomarqueurs ou bioindicateurs qualitatifs, et les bioindicateurs quantitatifs.

IV.2.1. Bioindicateur écologique

Un bioindicateur écologique peut se définir comme « un taxon ou un ensemble de plusieurs taxons qui, par ses caractéristiques qualitatives et/ou quantitatives, témoigne de l'état d'un système écologique et qui, par des variations de ses caractéristiques, permet de détecter d'éventuelles modifications de l'état de ce système » (Blandin 1986). Les bioindicateurs écologiques peuvent donc être utilisés pour mettre en évidence d'éventuelles perturbations rencontrées aux niveaux supérieurs d'organisation biologique tels que les populations, les peuplements et les communautés (présence/absence, abondance d'un ou plusieurs taxons). Cependant, ce type d'indicateur n'est pas spécifique d'un type de perturbation ou de contamination et traduit généralement une altération globale d'une biocénose sans que l'origine ou l'impact respectif des différentes sources de stress soient connu.

IV.2.2. Bioindicateur qualitatif ou biomarqueur

Un biomarqueur ou bioindicateur qualitatif peut se définir comme étant « un changement observable et/ou mesurable au niveau moléculaire, biochimique, cellulaire, physiologique ou comportemental, qui révèle l'exposition présente ou passée d'un individu à au moins une substance chimique à caractère polluant » (Lagadic et al. 1997). Les biomarqueurs constituent des outils permettant de détecter de manière relativement précoce une contamination chimique et évaluer les effets de différents contaminants aux niveaux infra-individuels et individuels (Depledge et al. 1993; Addison 1996). Les biomarqueurs (e.g., l'induction des métallothionéines, l'induction du système du cytochrome P450, Cajaraville et al. 2000; Perez et al. 2004) sont des outils sensibles utilisés dans les réseaux de surveillances, et permettent de détecter les effets biologiques de la contamination (McCarthy & Shugart 1990). Cependant, l'interprétation des résultats est généralement rendue difficile par l'influence

exercée par les fluctuations naturelles de nombreux facteurs biotiques et abiotiques sur les réponses observées (Cormier & Daniel 1994).

IV.2.3. Bioindicateur quantitatif

Un bioindicateur quantitatif est une espèce animale ou végétale dont l'analyse permet d'obtenir des informations intégrées dans le temps sur l'état de la contamination du milieu et sur la biodisponibilité des contaminants présents (Rainbow 1995). L'intérêt des bioindicateurs quantitatifs a été largement discuté par différents auteurs (Rainbow & Phillips 1993; Rainbow 1995) et la principale qualité d'un bioindicateur quantitatif est sa capacité de bioaccumuler un ou plusieurs contaminants. Le dosage des contaminants dans les tissus des espèces indicatrices vise dès lors à détecter et/ou à quantifier l'état de contamination du milieu. Si les espèces ciblées sont des espèces comestibles, elles peuvent être utilisées comme indicateur pour l'environnement et pour la santé humaine. Les bioindicateurs quantitatifs permettent de quantifier la fraction de la contamination biodisponible pour les organismes.

Un bioindicateur est défini comme « *un organisme qui peut-être utilisé pour quantifier les niveaux relatifs de pollution par les mesures des concentrations en contaminants dans ses tissus* » (Phillips 1990). Les méthodes basées sur l'utilisation de bioindicateurs pour estimer la qualité chimique du milieu côtier ne peuvent pas se faire avec l'ensemble des organismes marins. Il est donc nécessaire d'identifier et de sélectionner les espèces ou groupes d'espèces les plus aptes à pouvoir refléter la qualité de leur milieu.

IV.2.4. Critère de sélection d'un bioindicateur quantitatif

Les critères de sélection d'un organisme susceptible d'être utilisé comme bioindicateur de contamination ont été décrits par de nombreux auteurs (Phillips 1977b, 1990; Rainbow & Phillips 1993; Rainbow 1995). Notons, l'originalité de la définition d'Hopkin (1993) qui considère qu'un organisme bioindicateur pourra être utilisé avec succès s'il satisfait les '5R': « *Relevant, Reliable, Robuste, Responsive, Reproducible* », ou encore celle de de Kock & Kramer (1994), qui décrivent les critères de sélection des bioindicateurs selon les termes suivants « *Versatility, Practicability, Integrative ability, Consistency* ». Ces multiples définitions peuvent être résumées selon les critères énoncés ci-dessous, un bioindicateur doit :

-être **identifiable facilement** et sans ambiguïté au rang de l'espèce et **être une espèce dont la biologie et l'écologie sont bien connues;**

-être **abondant** et largement **distribué**, pour autoriser des prélèvements réguliers et en quantité suffisante pour être statistiquement exploitable tout en ne causant pas de préjudice aux populations en place;

-être facile à **récolter et à manipuler;**

-**avoir une durée de vie suffisamment longue et pouvoir être prélevé toute l'année** afin de pouvoir assurer un suivi dans le temps et prendre en compte la variabilité saisonnière;

-être **de taille suffisante** pour fournir la quantité de tissus nécessaire pour les analyses individuelles;

-être **résistant au stress** généré par les manipulations au cours de la récolte ou en laboratoire;

-être **tolérant aux variations des conditions naturelles de l'environnement**;

-être **sessile ou sédentaire** afin d'être représentatif d'une certaine localisation et d'intégrer les caractéristiques environnementales du site de prélèvement;

-être **de bon bioaccumulateur** des métaux étudiés.

Ces pré-requis ne sont pas entièrement suffisants pour valider le choix d'un bioindicateur. En effet pour comparer des échantillons provenant de différents sites, il faut s'assurer que les variables biotiques et abiotiques n'affectent pas les concentrations métalliques dans les organismes (Phillips 1980; Bryan et al. 1985). Ce dernier critère demeure le principal, et il a été clairement énoncé par Phillips (1990), « *all organisms in a survey exhibit the same correlation between their metal contents and those in the surrounding water, at all locations studied, under all conditions* » qui se traduit par « *tous les organismes dans une situation présentent la même corrélation entre leur contenu métallique et celui dans l'eau ambiante, à toutes les localisations étudiées et sous toutes les conditions* ».

IV.2.5. Organismes généralement utilisés

Selon les différents critères définis ci-dessus, le choix des organismes reste crucial. En pratique, il n'existe aucune espèce répondant à la totalité des critères cités précédemment. En effet, le choix des bioindicateurs se heurte aux problèmes de terrain. Les organismes sont donc sélectionnés selon l'ordre d'importance accordée à chaque critère, en tenant compte de la zone d'étude et des besoins. La sélection d'un seul bioindicateur ne permet pas de renseigner sur l'état global de contamination de l'écosystème. En effet, il a été montré qu'en l'absence de bivalves filibranches, la contamination en Cd n'aurait pas été mise en évidence

dans un site soumis aux apports par la Gironde (Miramand et al. 1999). Des espèces différentes ont généralement des distributions géographiques différentes, allant des rivages rocheux aux estuaires vaseux. Elles sont donc susceptibles d'accumuler les métaux à partir de différentes sources, et de représenter différentes fractions biodisponibles. Par exemple, dans l'estuaire de la Looe, l'Ag particulaire est plus facilement accumulé par les bivalves déposivores *Scrobicularia plana* et *Macoma balthica*, que par la moule *Mytilus edulis* et *a fortiori* par l'algue *Fucus vesiculosus* (Bryan et al. 1980). Ainsi, il n'existe pas un bioindicateur universel. C'est pour cette raison qu'il est fortement recommandé de travailler sur plusieurs espèces en parallèle : une algue, un bivalve filtreur et un déposivore afin d'estimer au mieux la biodisponibilité des métaux pour l'écosystème considéré. Les espèces les plus communément utilisées dans les programmes de biosurveillance sont les algues et les bivalves. En général, les macroalgues brunes sont les plus aptes à être utilisées comme bioindicateurs de part leur aptitude à fortement concentrer les métaux (Rainbow & Phillips 1993). De part leur physiologie, les algues accumulent les métaux principalement par voie dissoute (Bryan 1969, 1971; Phillips 1979), et elles seront le plus à même de représenter la contamination présente dans la fraction dissoute.

Les bivalves, dénommés « capteurs de contaminants », sont utilisés en complément des algues. En effet, de part leurs particularités écologiques et leur mode d'alimentation basé sur la filtration de larges quantités de matières particulaires, les bivalves 'filtreurs' sont capables de refléter la contamination des métaux présents sous forme dissoute et particulaire (sédiments, phytoplancton, matières en suspension). Les bivalves 'fouisseurs', quant à eux ingèrent du sédiment en se nourrissant, et seront le plus à même de refléter les métaux associés à la fraction sédimentaire. De plus, le caractère sédentaire des bivalves, dû à leurs particularités biologiques, est fort utile pour détecter la localisation précise de sources de contaminants. Leur cycle de vie de quelques mois à plusieurs années permet de suivre les changements temporels des contaminants sur une échelle de temps relativement longue, et d'intégrer les effets épisodiques des variations des contaminants. Les espèces de la famille des Mytilidae, de part leur abondance et leur large distribution dans plusieurs régions du monde, leur forte tolérance aux stress naturels opérant dans la zone tidale (Davenport 1979; de Kock & Kuiper 1981) sont souvent proposées comme bioindicateurs. Cette aptitude exceptionnelle des bivalves et des algues à la bioaccumulation a donc été mise à profit dans les années 1960-70 par la mise en place dans des programmes de surveillance des contaminants, appelé « biomonitoring ».

V. BIOMONITORING: PROGRAMME DE SURVEILLANCE DES CONTAMINANTS

Le suivi de l'état de contamination du milieu marin à l'aide d'espèces bioindicatrices est nommé « biomonitoring ». Le biomonitoring ou « biosurveillance » des contaminants constitue une partie de la recherche en écotoxicologie et permet l'obtention de différentes informations (e.g. le degré et les variations géographiques et temporelles des concentrations en contaminants). Il peut être réalisé de deux façons : (1) la biosurveillance passive (les organismes sont prélevés directement dans le ou les sites étudiés parmi les populations en place) et (2) la biosurveillance active (les organismes sont prélevés dans une population unique puis transplantés dans les sites à étudier; ils y sont généralement maintenus de quelques semaines à quelques mois avant d'être analysés).

V.1. SURVEILLANCE EN MILIEU TEMPERE

V.1.1. Mussel Watch

Historiquement, un des premiers dispositifs destiné à évaluer la qualité de l'eau de mer a été le « Mussel Watch » aux Etats-Unis (e.g., Goldberg 1975). Débuté vers 1965 sous le contrôle de l'Environmental Protection Agency (EPA), il est actuellement pris en charge par la « National Oceanographic and Atmospheric Administration » (NOAA). A partir de 1992, l'utilisation du Mussel Watch s'est étendu en Amérique du Sud et en Amérique centrale (Tripp et al. 1992). L'espèce utilisée varie selon la localisation géographique : la Moule bleue commune *Mytilus edulis* et la moule de Californie *Mytilus californianus* sur l'Atlantique nord et la côte du Pacifique jusqu'à l'Alaska, l'Huître américaine *Crassostrea virginica* dans le Golfe du Mexique et l'Atlantique sud, l'huître hawaïenne *Ostrea sandvicensis* à Hawaï (Cantillo 1991). Les mollusques sont collectés systématiquement pendant l'hiver sur environ 200 sites, puis stockés avant d'être analysés. Les concentrations de plusieurs contaminants (métaux lourds, molécules organochlorées), et plus récemment divers paramètres biochimiques sont déterminés. D'après les résultats obtenus, la répartition de la plupart des contaminants est relativement uniforme le long des côtes des États-unis, avec des zones plus fortement contaminées autour des zones industrielles. L'extension du Mussel Watch a atteint le bassin Méditerranéen, avec la mise en place d'un « Mediterraneen Mussel Watch » (CIESM 2002), et plus particulièrement le littoral atlantique marocain, où la moule *Mytilus galloprovincialis*

est utilisé comme bioindicateur quantitatif de la contamination par le Cd, le Cu, le Mn et le Zn (Chafik et al. 2001). Désormais, le principe du Mussel Watch est donc utilisé à grande échelle par de nombreux pays pour dresser une cartographie de la contamination marine et suivre son évolution.

V.1.2. RNO et RINBIO en France

Basé sur le concept du Mussel Watch, l'Institut Français de Recherche pour l'Exploitation de la Mer (I.F.R.E.MER) a crée le Réseau National d'Observation (R.N.O.) en 1974. Il permet d'évaluer, le long du littoral français, les concentrations et les tendances de plusieurs contaminants (métaux, organochlorés, hydrocarbures aromatiques polycycliques ou H.A.P.) dans différents compartiments (eau de mer, matière vivante, sédiments). Concernant le compartiment biologique, des moules et des huîtres (*Mytilus edulis, Mytilus galloprovincialis* et *Crassostrea gigas*) sont échantillonnées 2 fois par an en métropole sur 90 points de prélèvements (e.g., RNO 2004, 2005). Le Réseau INtégrateur BIOlogique, RINBIO, développé par l'IFREMER depuis 1996 a pour objectif d'évaluer les concentrations chimiques et radiochimiques dans chaque unité du référentiel géographique du Schéma Directeur d'Aménagement et de Gestion des Eaux (SDAGE) du bassin méditerranéen corse (RINBIO 2001). Dans le cadre du réseau RINBIO, la technique et les avantages de la biosurveillance active ont été utilisés. Les organismes y sont généralement maintenus de quelques semaines à quelques mois avant d'être analysés, ils peuvent alors accumuler les contaminants jusqu'à un état de pseudo-équilibre. Les transplants d'organismes permettent de sélectionner les stations voulues, même si les organismes n'y sont pas naturellement présents, et la période d'exposition est connue.

V.2. SURVEILLANCE EN MILIEU TROPICAL

Si de grands efforts ont été réalisés dans les milieux tempérés, avec le Mussel Watch aux Etats-Unis et son extension dans d'autres pays tempérés, très de peu de choses ont été mises en place dans les milieux tropicaux. Par ailleurs, dans le domaine de l'écotoxicologie des métaux, la plupart des informations publiées concernent les écosystèmes marins tempérés et assez peu de données s'intéressent aux régions tropicales (Lacher & Goldstein 1997). Malgré tout depuis quelques années, un intérêt certain est porté aux récifs coralliens. Les récifs coralliens supportent une grande diversité de vie, et jouent un rôle essentiel pour les populations locales (e.g. pêche, tourisme) sont en effet très sensibles aux perturbations anthropiques.

V.2.1. Surveillance de l'état de santé des récifs coralliens

L'état de dégradation des récifs coralliens est préoccupant, et de nombreux programmes (International Coral Reef Initiative I.C.R.I.; Coral Reef Initiative for South Pacific C.R.I.S.P., Global Coral Reef Monitoring Network G.C.R.M.N., United Nations Environment Programme U.N.E.P) sont nés dans le but d'évaluer l'état de santé des récifs coralliens à travers le monde afin d'améliorer leur gestion et leur protection. Cependant, cette surveillance biologique des zones coralliennes est basée soit sur des approches écologiques (mesure de la biodiversité, de couverture algales et coralliennes); soit sur l'étude des peuplements de poissons et/ou invertébrés; soit sur la structure des communautés. A titre d'exemple, la présence des poissons papillons *Chaetodon* spp. est utilisé comme indicateur de l'état santé d'un récif corallien (Öhman et al. 1998). Il est aussi possible d'utiliser en parallèle plusieurs approches écologiques. L'étude de Dahl (1981) prévoyait des comptages de poissons, la description du substrat et des formes coralliennes, la mesure de la couverture algale et corallienne, afin de mettre au point un système de surveillance des récifs coralliens situés autour de la ville de Nouméa et sujets aux rejets urbains. Cependant, de telles approches fournissent des informations uniquement sur l'état de santé globale du récif, et ne donnent pas d'information sur la présence et/ou les effets d'éventuelles contaminations chimiques.

V.2.2. Surveillance des contaminants, un programme rare

Pour ce qui est de la contamination chimique, les programmes de surveillance sont rares avec une notable exception pour l'étude de l'eutrophisation dans les systèmes récifo-coralliens (Bell 1991, 1992; Larkum & Steven 1994; Koop et al. 2001). Une synthèse de Phillips (1991) sur les métaux en domaine tropical met l'accent sur le faible nombre d'études concernant les impacts des métaux sur les écosystèmes tropicaux, par rapport aux travaux réalisés en milieu tempéré, alors que ces écosystèmes présentent une forte vulnérabilité. Parmi les travaux publiés sur l'écotoxicologie des métaux dans le milieu marin tropical, la plupart se sont intéressés à l'aspect descriptif des concentrations en métaux dans l'eau, les sédiments et les organismes vivants (e.g., Denton & Burdon-Jones 1986b; Gibbs & Guerra 1997). Pour ces derniers, l'intérêt semble avoir été principalement porté aux mollusques bivalves -huîtres et moules- (e.g., Phillips & Muttarasin 1985; Peerzada & Dickinson 1988) et aux coraux bâtisseurs de récifs (e.g., Gusmán & Jimenez 1992; Haynes & Johnson 2000). Quelques études ponctuelles portent également sur des poissons, des crustacés, des algues, des phanérogames marines et des échinodermes (e.g., Nienhuis 1986; Jones et al. 2000).

Cependant, à l'exception de quelques travaux (e.g., Klumpp & Burdon-Jones 1982; Blackmore & Wang 2003), les organismes étudiés ont été sélectionnés sur des critères pratiques (e.g., présence) plutôt que sur des critères garantissant leur validité comme bioindicateurs.

Actuellement, rares sont les pays sous ces latitudes où de véritables réseaux de surveillance du milieu marin ont été développés. En effet, comme nous l'avons vu précédemment le choix des espèces demeure crucial. Dans les milieux tempérés, la moule *Mytilus edulis* est utilisée comme bioindicateur pour le « Mussel Watch ». Or, en milieu tropical, il n'existe pas de moules qui puisse se substituer à la moule *Mytilus edulis* utilisée comme bioindicateur en domaine tempéré et des études visant à identifier d'autres organismes tropicaux susceptibles d'être utilisés comme bioindicateur se sont développées. Récemment, le Mussel Watch a été étendu dans la région Asie/Pacifique sous le nom de « Asia-Pacific Mussel Watch » (APMW) (UNU 1994). Les espèces susceptibles d'être utilisées comme bioindicateur étaient du genre *Saccostrea, Crassostrea* et *Perna*. D'août 1995 en juillet 1998, le projet APMW a été développé pour mesurer les concentrations des métaux et des composés organiques dans les sédiments, les tissus d'huîtres et autres coquillages prélevés dans 17 sites côtiers et estuariens aux alentours de Taiwan (Jeng et al. 2000). Dans le Sud-Est asiatique, la moule verte *Perna viridis* a été identifiée comme bioindicateur potentiel des métaux (Phillips 1985). L'extension du R.N.O. français au littoral de certains départements d'Outre-mer (e.g. Guadeloupe, Martinique, La Réunion) est également en cours, et cette démarche est bien évidemment renforcée dans le contexte de l'application de la directive cadre sur l'eau de la Commission Européenne. Aux Antilles, la surveillance des contaminants dans les organismes utilise une espèce très commune, l'huître *Isognomon alatus* (RNO 2005). En Polynésie française, la réponse physiologique de la moule tropicale *Modiolus auriculatus* aux contaminants organochlorés est présenté comme un outil permettant l'évaluation de leur toxicité et leur suivi dans l'environnement marin (Bourdelin 1996). Néanmoins, ces essais ne sont pas encore finalisés et un programme de biosurveillance, utilisé en routine dans toute la zone tropicale comme le Mussel Watch dans les zones tempérées, reste encore difficilement envisageable aujourd'hui, notamment de part les problèmes d'instabilité des gisements naturels des espèces utilisées (e.g. *Isognomon alatus,* RNO 2005).

De plus, les informations disponibles sur le milieu marin tropical concernent préférentiellement certaines régions du monde. En effet, la majorité des travaux publiés concernent la Baie de Hong-Kong en mer de Chine (e.g., Chong & Wang 2000a; Blackmore

& Wang 2003), le sud-est asiatique (Blackmore 1998), l'Amérique latine (Paez-Osuna & Tron-Mayen 1996; Alho & Vieira 1997; Gibbs & Guerra 1997) et la Grande Barrière de corail au nord-est de l'Australie (e.g., Denton & Burdon-Jones 1986a; Jones et al. 2000; Olivier et al. 2002). Au contraire, quasiment aucune information n'est disponible pour les côtes africaines et seules quelques études ponctuelles se sont intéressées à la région indopacifique (Nienhuis 1986; Lim et al. 1995) et plus particulièrement au site d'Hawaii (Hunter et al. 1995). Ce manque d'information se fait sérieusement ressentir dans plusieurs régions, en particulier celles où des problèmes environnementaux évidents existent. C'est notamment le cas de la Nouvelle-Calédonie où les apports en métaux générés par les activités minières en plein développement imposent de prendre des dispositions pour surveiller et gérer la qualité de l'environnement (Labrosse et al. 2000).

VI. CONTEXTE LIE A LA NOUVELLE-CALEDONIE

VI.1. GENERALITES

VI.1.1. Géographie

Le « Caillou » comme la dénomme les habitants de la Nouvelle Calédonie a été découvert à la fin du XVIII^{ème} siècle par le capitaine Cook (1774). La Nouvelle-Calédonie se situe dans le Pacifique Sud (entre 19° et 23° de latitude sud et entre 158°et 172° de longitude est) (Fig. 4).

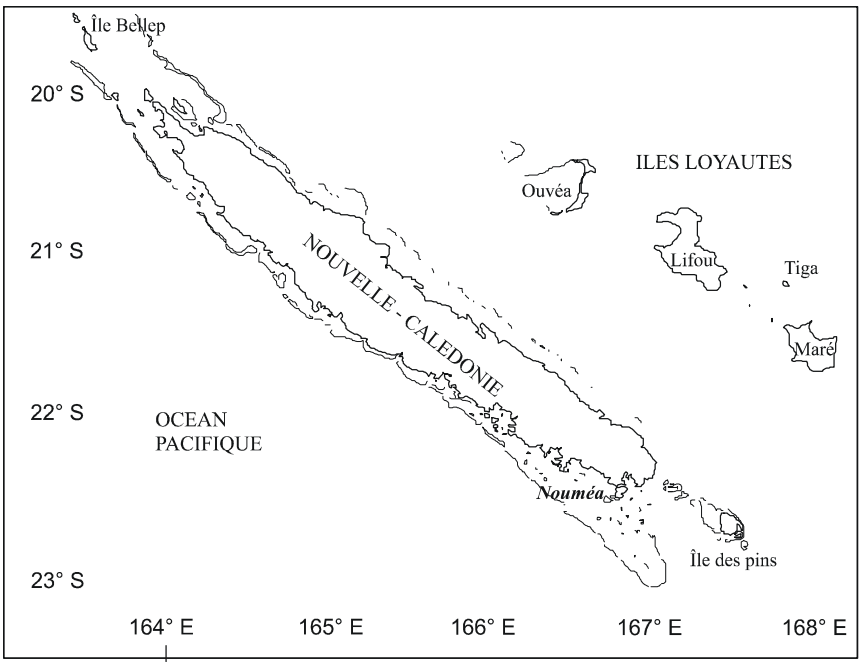

Figure 4. Carte de la Nouvelle Calédonie.

L'île principale, « la Grande Terre », est la région la plus riche d'un point de vue démographique de la Nouvelle-Calédonie. Elle est divisée dans la longueur par un massif montagneux appelé « Chaîne Centrale » qui sépare l'île en 2 régions très différentes :

- la Côte Est, humide, exposée aux alizés, avec une végétation typique des zones tropicales humides;
- la Côte Ouest, plus sèche et tempérée, avec une plaine côtière couverte d'une végétation de type savane.

La flore et la faune de la Nouvelle Calédonie sont uniques avec un fort taux d'endémisme (75% des 3 500 espèces de plantes) ; on y a pour l'instant recensé 4 300 espèces d'animaux terrestres, 1 000 espèces de poissons et 6 500 espèces d'invertébrés marins. Entourée par une barrière de corail de 1 600 km de long, la Nouvelle-Calédonie compte également l'un des plus vastes lagons au monde.

VI.1.2. Climatologie

Situé au niveau de la ceinture intertropicale, la Nouvelle Calédonie est soumise à un climat tropical océanique. Deux saisons majeures se distinguent, la saison chaude de décembre à mars et la saison fraîche de juin à août, entrecoupées par deux saisons de transition. La saison chaude correspond à la période de développement des dépressions tropicales ou des cyclones. Le climat est caractérisé par une pluviométrie forte et discontinue pendant la saison fraîche. Les alizés soufflent toute l'année de façon modérée à forte, entre 15 et 20 nœuds, dans un secteur compris entre l'est à sud-est. La température moyenne est de 22-24°C.

VI.1.3. Géologie

Au niveau géologique, l'abondance exceptionnelle des roches ultrabasiques donne son originalité à la chaîne de la Grande Terre. Lors de la collision entre plaques, génératrice du dernier plissement, un vaste feuillet de ce manteau est remonté et a été poussé par-dessus les autres terrains. L'érosion l'a disséqué en plusieurs massifs. Ces roches ultrabasiques, provenant du manteau, présentent dès leurs origines une richesse en métaux de transition, notamment en Ni. Ces roches appelés péridotites contiennent de forte teneur en métaux (0,3% Ni) et occupent plus d'un tiers de la superficie du Territoire. La formation des *péridotites* correspond à l'étape primaire d'enrichissement en métaux du sous-sol. Cet enrichissement a pu se poursuivre par une seconde étape de formation des *serpentines*. Sous l'action de facteurs tectoniques, les péridotites subissent un charriage et une hydratation partielle des

silicates. Sous l'action climatique, elles sont lessivées en profondeur entraînant un départ des alcalins et alcalino-terreux, et d'une partie de la silice et enfin une migration des métaux de transition. D'où une concentration importante de ces métaux dans une partie du profil d'altération, nommés *saprolites* ou *garniérites*, où les teneurs en Ni peuvent atteindre 2 à 3%. De part cette évolution géochimique, est née l'existence de zones géographiques très riches en Co, Cr, Fe et Ni. L'originalité des sols de Nouvelle Calédonie a suscité l'intérêt des chercheurs et des industriels par leur richesse exceptionnelle en hydroxyde de Fe et en métaux de transition.

VI.2. ACTIVITES MINIERES

La présence en Nouvelle-Calédonie de minerai de Ni ou « or vert » fut découvert en 1774 par Jules Garnier. Depuis plus d'un siècle, la richesse des sols calédoniens en métaux -Ni, mais aussi Ag, Au, Co, Cu, Fe, Mn et Pb- a été exploitée par les industriels. Actuellement, l'essentiel de l'activité minière du Territoire concerne le Ni et, secondairement le Co. La Nouvelle Calédonie est aujourd'hui le troisième producteur de Ni à l'échelle mondiale (50 000 tonnes/an) et elle détiendrait la plus grande réserve de Ni dans ses sols (25% des réserves mondiales). L'économie calédonienne est essentiellement fondée sur l'exploitation des gisements de Ni qui représente 95% de ses exportations.

Le Ni se présente à l'état naturel sous deux formes : les minerais sulfurés, dont les gisements les plus importants se situent au Canada, Australie et Afrique du Sud, et les minerais oxydés, qui proviennent essentiellement du Pacifique Sud (Nouvelle Calédonie, Indonésie, Philippines) et d'Amérique latine (Cuba, Brésil). Les minerais oxydés se présentent sous deux formes :

- les garniérites (minerais oxydés silicatés), dont la teneur en Ni varie entre 2-3%,
- les latérites (minerais latéritiques) avec des teneurs plus faibles (1-1,6%).

La principale méthode d'extraction utilisée en Nouvelle-Calédonie consiste à défricher les sommets des collines et à y creuser des mines à ciel ouvert. Elle commence par un décapage de la cuirasse ferralitique, puis des latérites à faible teneur en Ni (latérites rouges et jaunes) pour atteindre la garniérite qui constitue le minerai exploité (Fig. 5).

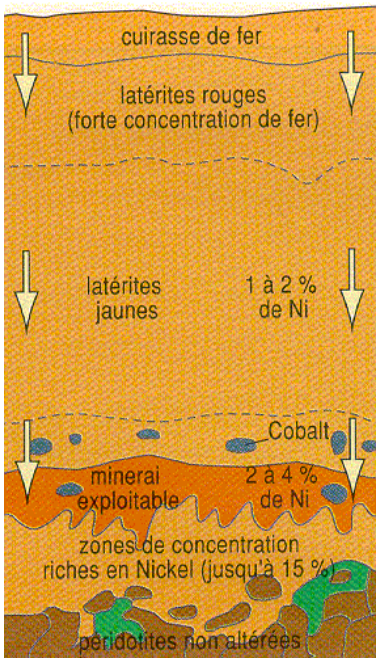

Figure 5. Profil vertical de l'altération de la roche mère du sol néo-calédonien riche en péridotites.

L'usine de Doniambo de la Société Le Nickel (S.L.N.) est aujourd'hui le principal site de traitement du minerai de Ni en Nouvelle Calédonie, avec une capacité de production de 50 000 tonnes par an. Cette usine utilise pour le traitement du minerai un procédé traditionnel de pyrométallurgie. Cette technique est basée sur la fusion du minerai à forte teneur en Ni (2-3%) dans des fours. La position géographique de la S.L.N. en bord de la Grande Rade implique des rejets dans le lagon de formes liquides, dues aux eaux de refroidissement utilisées dans le processus industriels; et de formes solides, liés au déchargement des minéraliers, aux scories et aux poussières atmosphériques. Le processus d'extraction à ciel ouvert n'est pas sans danger pour l'environnement et plusieurs conséquences écologiques non négligeables résultent de ce procédé (Bird et al. 1984; Labrosse et al. 2000) :

- la déforestation, la destruction d'habitats terrestres et la disparition d'espèces endémiques,
- l'accélération du lessivage et de l'érosion des sols et, de là, l'augmentation de la quantité de particules métallifères charriées par les rivières,
- l'augmentation de la turbidité et de la sédimentation dans le lagon,
- l'augmentation des apports métalliques dans la zone côtière.

Récemment, une nouvelle méthode d'extraction du Ni et du Co a été développée par la société minière Goro-Nickel (branche d'Inco Limited) qui projette d'exploiter les gisements de minerai latéritique de Ni et de Co de la région de Goro, situé dans la province Sud de la Nouvelle-Calédonie (Fig. 6A, 6B).

-A- -B-

Figure 6. -A- Terres latéritiques au Sud de l'île et -B- Vue du site de l'usine Goro-Nickel.

Ce nouveau procédé permettrait d'utiliser des minerais à faible teneur en Ni (latérites) peu exploitées jusqu'alors. Le procédé de type hydrométallurgique est basé sur la ***lixiviation acide sous pression (LAP)***. Cette méthode LAP consiste en une mise en solution des métaux contenus dans le minerai, suivi d'une extraction par solvant pour séparer le Ni et le Co de la solution acide. En 1999, GNi a construit et mis en service une usine pilote à l'échelle 1:35ème. Cette dernière avait pour principal objectif de démontrer la viabilité du procédé hydrométallurgique et de recueillir des données pour la conception de l'usine commerciale (Goro-Nickel 2001). La construction d'une usine est maintenant en cours et devrait permettre à partir de 2007 une production de 54 000 t de Ni et 5 400 t de Co par an. Le traitement hydrométallurgique des latérites permet de valoriser le Ni et le Co présent dans le minerai, à la différence du procédé pyrométallurgique axé sur la seule valorisation du Ni. La récupération du Co dans le minerai est un élément important de rentabilité : la vente de celui-ci devrait représenter environ 25% des recettes totales (Goro-Nickel 2001).

Les conséquences environnementales de ce nouveau projet d'extraction par lixiviation acide sous pression (LAP) préoccupent naturellement la population, les autorités, les organisations écologiques néo-calédoniennes et donc le groupe industriel. En effet, les effluents de l'usine présenteront des concentrations élevées en métaux autres que le Ni et le Co (retenus à 90 % par le procédé industriel), notamment pour le Mn qui présente des concentrations supérieures

aux normes autorisées par l'*Arrêté 1542-99/PS* de la Nouvelle-Calédonie et de l'*Arrêté du 2 février 1998* imposant une limite de concentrations des rejets de 1 mg Mn l^{-1}.

Élément	Effluent (µg l^{-1})	Concentration attendue dans le canal de la Havannah à 20 m du diffuseur (µg l^{-1})	Eau de mer du canal de la Havannah (µg l^{-1})	Norme pour l'eau de mer CCC* (µg l^{-1})
As	50	0.05	1.5	36
Cd	100	0.1	0.02	8,8
Cr	50	0.05	0.3	50,0 (Cr VI)
Cu	20	0.02	0.3	3.1
Mn	100000	100	3	
Ni	500	0.5	0.32	8.2
Barrière	50	0.05	1.5	81

*USEPA (2004) National Recommended Water Quality Criteria. L'un des critères de qualité de l'eau d'après ses effets prévu d'après les lignes directrices de l'US EPA est le Critère de concentration continue (CCC), qui est une estimation de la concentration maximale d'une matière dans les eaux de surface à laquelle une communauté aquatique peut être exposée indéfiniment sans subir un effet indésirable.

Tableau 4. Concentrations en éléments attendues dans les effluents de l'usine pilote GNi et dans le canal de la Havannah (Goro-Nickel 2003).

Les apports accrus de ces éléments métalliques sont susceptibles d'induire des effets directs ou indirects sur les organismes et, ceci, à plus forte raison dans un système lagonaire partiellement isolé de l'océan par une barrière récifale.

VI.3. IMPACT DES METAUX SUR L'ENVIRONNEMEMT

Au terme de ces quelques paragraphes, le paradoxe calédonien apparaît dans toute son ampleur : l'écosystème terrestre et marin de la Nouvelle-Calédonie possède une extrême richesse en terme de biodiversité mais les activités minières, principales ressources économiques du Territoire, constituent la source majeure de perturbation et de contamination de l'environnement. Une importante question se pose : comment concilier la richesse écologique de l'île avec sa richesse économique ? Le développement économique de la Nouvelle Calédonie passe par la cohabitation du développement des activités minières du Ni (50 000 de tonnes de ce métal ont été exportées en 2003) et par la préservation d'une forte biodiversité (marine et terrestre). Dans ce contexte, le lourd déficit d'informations

scientifiques sur les effets des apports en métaux sur l'environnement doit être comblé de toute urgence.

VI.3.1. Au niveau terrestre

Dans le domaine botanique, les travaux réalisés montrent la capacité d'adaptation de la flore dans les terrains miniers du Sud, très riches en métaux de transition (Jaffré et al. 1979b; Boyd et al. 1999). Certaines espèces sont décrites comme des organismes hyper-accumulateurs de Ni, organismes dont les concentrations sont supérieures à 1000 µg de Ni g^{-1} de poids sec (e.g., *Sebertia acuminata*, Jaffré et al. 1976 ; *Geissois* spp., Jaffré et al. 1979a et *Phyllanthus* spp., Kersten et al. 1979). De telles concentrations en Ni, fatales pour la plupart des phanérogames, soulèvent des questions intéressantes sur la physiologie des plantes endémiques à la Nouvelle Calédonie, et à leur capacité d'adaptation dans un environnement normalement toxique.

VI.3.2. Au niveau marin

Contrairement au milieu terrestre où un grand nombre d'études a été fait tant au niveau géologique que botanique, une étude bibliographique des travaux réalisés souligne la méconnaissance scientifique dans le domaine des relations entre les exploitations minières intenses dans le Pacifique insulaire tropical et l'environnement marin (Labrosse et al. 2000). Des études récentes sur la géochimie des sédiments permettent de dresser un premier bilan en termes de 'géoaccumulation', de biodisponibilité potentielle et d'origine des flux de Co, Cr, Cu, Fe, Mn, Ni et Zn (Breau 1998; Fernandez et al. 2002b; Fernandez et al.). Cependant, ces études ne constituent qu'une étape dans la compréhension des mécanismes de transfert qui nécessite la prise en compte de l'incorporation des métaux dans les organismes marins. Jusqu'en 2000, les seules données disponibles pour la Nouvelle Calédonie provenaient de mesures de Fe et Ni chez les ascidies (Monniot et al. 1994) et de 12 métaux chez le nautile *Nautilus macromphalus* (Bustamante et al. 2000) qui indiquaient des concentrations pouvant être considérées comme élevées. Au-delà du seul intérêt local, la Nouvelle-Calédonie peut-être en outre considérée comme une « zone laboratoire » pour le Pacifique Sud car la plupart des biotopes lagonaires s'y trouvent représentés et certaines espèces présentent une vaste répartition géographique (PNEC 2003). En Nouvelle Calédonie, l'utilisation d'organismes marins comme bioindicateurs des activités minières n'est pas une idée récente. Le projet NORCAL en 1981 proposait des espèces d'oursins et d'holothuries comme indicateurs en raison de leur forte sensibilité aux conditions du milieu (Binet & Boëly 1981). Mais le projet n'a pas abouti. La principale difficulté résidait dans le choix des espèces. L'intérêt d'une telle

approche est évident pour la Nouvelle-Calédonie mais constituera plus largement une base de référence pour les pays insulaires du Pacifique. Dans ce contexte, et dans le cadre du Programme National Environnement Côtier (PNEC, France), une première étape importante a été franchie avec la thèse de Breau (2003) qui a identifié des espèces benthiques bioaccumulatrices pour obtenir un premier bilan sur le degré de contamination du lagon. Les travaux réalisés à ce jour constituent une première étape de prospection, caractérisant la présence et l'abondance des espèces animales et végétales dans 84 stations du lagon Sud-Ouest. Treize espèces (8 invertébrés et 5 algues) se sont révélées être largement distribuées dans le lagon. Les concentrations en différents métaux (Al, Co, Cr, Cu, Fe, Mn, Ni, Zn) ont ensuite été déterminées dans ces 13 espèces prélevées dans 6 sites intertidaux ou subtidaux soumis à des conditions de contamination supposées contrastées (4 sites plus ou moins directement soumis aux effluents de rivières drainant des régions minières et 2 sites « contrôles » isolés des sources d'apports anthropiques). Les données métalliques conjuguées aux données écologiques (répartition et abondance des taxa) ont permis de sélectionner quatre espèces présentant un bon potentiel pour être utilisées comme bioindicateurs de contamination métallique en milieu lagonaire (Breau 2003). Il s'agit en l'occurrence :

- des huîtres *Malleus regula* et *Isognomon isognomon*

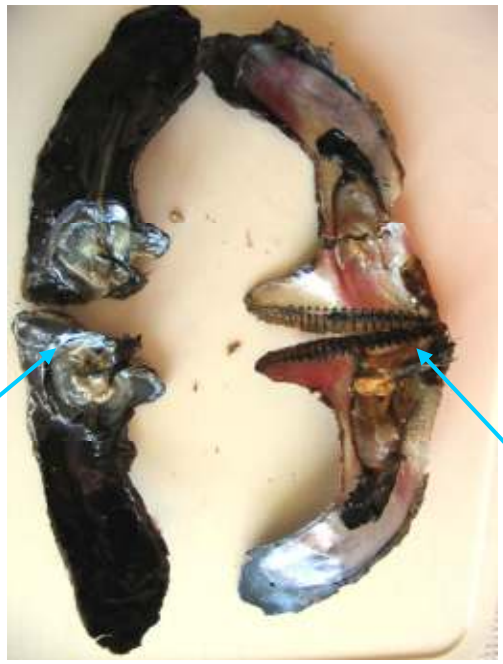

Figure 7. Les huîtres *Malleus regula* et *Isognomon isognomon.*

- du Veneridae *Gafrarium tumidum*,

Figure 8. Le clam *Gafrarium tumidum.*

- de l'algue brune *Lobophora variegata*,

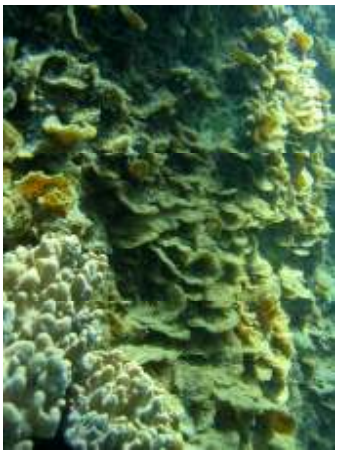

Figure 9. L'algue brune *Lobophora variegata.*

Les huîtres *I. isognomon* et *M. regula* partagent la même niche écologique (Yonge 1968). Elles vivent sur ou sous les rochers, dans les crevasses ou les débris de coraux, solidement fixés par leur byssus au substrat dur. Ces 2 espèces se ressemblent assez fortement et leur identification *in situ* peut parfois poser certains problèmes. Cette identification est toutefois plus aisée sur des individus désolidarisés de leur substrat : la forme de la charnière des valves ou la nacre sur la face interne des valves (sur des spécimens sacrifiés) sont des caractères taxonomiques univoques pour discriminer les 2 espèces (Yonge 1968). Les huîtres *Isognomon* spp. (*Isognomon isognomon* et *Isognomon alatus*) avaient déjà été proposées et utilisées comme bioindicateurs dans le vaste programme de Mussel Watch (Cantillo 1991).

La coque *Gafrarium tumidum* est un Verenidae qui vit enfoui dans les sédiments ; elle apprécie particulièrement les vasières. Le clam *Gafrarium tumidum* appelé plus communément « grisette » est une espèce comestible en Nouvelle Calédonie. Elle est récoltée en zone intertidale, notamment en baie de Dumbéa, Plage d'Ouano et même en Grande Rade.

La récolte de cette espèce est commercialisée localement d'une façon modeste. L'intérêt d'avoir une telle espèce dans un programme de biosurveillance apparaît en tant qu'indicateur de santé publique. La plupart des pays ont adopté des réglementations pour encadrer la production et la consommation de coquillages. De part la comestibilité de la grisette en Nouvelle Calédonie, la surveillance des concentrations des métaux dans cet organisme est à même de déceler un problème de contamination qui pourra avoir des répercussions au niveau de l'homme.

L'algue brune *Lobophora variegata* appartient à la classe des Fucophycées, ordre des Dictyotales, famille des Dyctyotaceae. Elle est commune sur les rochers et sur les coraux dans les lagons et sur les pentes des récifs, de la surface à environ 10 mètres de profondeur. On la trouve également dans les baies, où elle colonise les substrats rocheux ou coralliens morts.

Ainsi, l'algue brune *L. variegata*, les huîtres *I. isognomon* et *M. regula* et le clam *G. tumidum* ont été sélectionnées d'après les travaux de Breau (2003) en tant qu'espèces potentiellement intéressantes au titre de bioindicateurs de la contamination minière en Nouvelle Calédonie.

OBJECTIFS

Dans les états insulaires du Pacifique, le développement de l'industrie minière et le développement urbain sont responsables de contaminations métalliques importantes et durables des systèmes côtiers en général et lagonaires en particulier (Laws 1993; Zann 1994). Même si les données écotoxicologiques relatives au lagon de Nouvelle-Calédonie sont rares, la contamination du lagon néo-calédonien issus de l'exploitation minière (Co, Cr, Ni, Mn) mais aussi des activités domestiques, urbaines et agricoles (Cu, Zn) est aujourd'hui un fait avéré (Labrosse et al. 2000; Breau 2003). Dans le contexte actuel de développement industriel et avec l'apparition d'un nouveau procédé de traitement hydrométallurgique basé sur l'extraction du Ni et du Co par lixiviation à l'acide sulfurique, il est devenu urgent de disposer d'un programme de biosurveillance de l'état de la contamination des eaux lagonaires de Nouvelle-Calédonie. D'après les concentrations métalliques mesurées dans différents organismes tropicaux du lagon, quatre espèces locales répondant aux principaux critères de bases d'un bioindicateur (Phillips 1990; Rainbow & Phillips 1993) ont été sélectionnées au titre de candidat bioindicateur de l'état de la contamination des eaux lagonaires de Nouvelle-Calédonie. Il s'agit en l'occurrence de l'algue brune *Lobophora variegata*, du clam *Gafrarium tumidum* et de deux huîtres, *Isognomon isognomon* et *Malleus regula*.

La présente recherche doctorale avait pour objectif principal de caractériser le potentiel bioaccumulateur des quatre espèces cibles sélectionnées en laboratoire et *in situ* afin d'estimer si elles peuvent constituer des bioindicateurs fiables de la contamination métallique des eaux côtières du lagon néo-calédonien. L'utilisation du nouveau procédé de lixiviation va entraîner une solubilisation des métaux présents dans le minerai qui ne sera pas spécifique au Ni. Ainsi il est probable que des rejets liquides métallifères enrichis en métaux non exploités soient déversés dans l'environnement. Ainsi, au cours de ce travail, les contaminants considérés sont les métaux exploités ou en phase d'exploitation par l'industrie minière (Ni et Co) et les éléments présents en quantités non négligeables dans les rejets miniers issus de ces industries (Ag, As Cd, Cr, Cu, Mn et Zn -l'Ag quant à lui est un traceur bien connu des activités urbaines-).

Dans une première partie, la bioaccumulation des contaminants dans les quatre organismes (*L. variegata*, *G. tumidum*, *I. isognomon* et *M. regula*) a été étudiée en condition contaminante contrôlée. Les principaux facteurs biotiques/abiotiques pouvant influencer la bioaccumulation des métaux dans ces organismes ont été pris en considération : la taille des organismes, la concentration métallique et la voie d'accumulation. L'objectif était d'étudier l'influence de

ces différents paramètres sur la bioaccumulation des métaux afin de déterminer les conditions limites dans lesquelles les organismes peuvent être utilisés fiablement en tant qu'espèces bioindicatrices.

Les conditions contaminantes en milieu naturel étant plus complexes que celle reproduites en laboratoire de part la variabilité des facteurs environnementaux (e.g. disponibilité de la nourriture, particules en suspension), la deuxième partie de ce travail s'est attachée à étudier la valeur bioindicative des organismes sélectionnés *in situ*. Deux approches de l'utilisation des bioindicateurs (la biosurveillance active et passive) ont été considérées dans cette étude. Ainsi, dans un premier temps, la variation géographique des concentrations en contaminants dans l'algue *L. variegata*, le clam *G. tumidum* et l'huître *I. isognomon* a été étudiée afin d'estimer l'intérêt de suivre l'évolution des concentrations en contaminants dans les espèces résidentes pour une biosurveillance passive de la contamination. Ensuite, des transplantations croisées d'organismes issus de sites non contaminés et de sites contaminés ont été réalisées afin de caractériser la dynamique des processus d'accumulation et d'élimination des espèces sélectionnées et de déterminer si les espèces transplantées peuvent fournir une information fiable en terme de représentativité de l'état de contamination du site dans lequel elles sont transplantées.

Le but ultime de ce travail est de proposer une démarche permettant d'évaluer l'ampleur de la contamination environnementale d'origines minières, domestiques, agricoles ou urbaines le long des côtes calédoniennes à l'aide d'organismes marins locaux par une biosurveillance passive des populations résidentes et/ou par une biosurveillance active à l'aide d'organismes transplantés.

PREMIERE PARTIE : CARACTERISATION DU POTENTIEL BIOACCUMULATEUR DES ORGANISMES EN LABORATOIRE

Dans cette première partie, l'objectif principal était de caractériser les cinétiques d'accumulation et d'élimination des contaminants en conditions contrôlées de laboratoire dans l'algue brune *Lobophora variegata*, les huîtres *Isognomon isognomon* et *Malleus regula*, et le clam *Gafrarium tumidum.* La bioaccumulation des contaminants étant affectées par de nombreux facteurs biotiques/abiotiques, l'influence de certains facteurs biotiques/abiotiques sur la bioaccumulation des organismes a été étudiée afin d'estimer les conditions optimales dans lesquelles les organismes pourraient constituer des espèces bioindicatrices fiables de la contamination métallique. Il s'agit de l'influence de la taille des organismes, de la voie d'accumulation (eau de mer, sédiments, nourriture) et de la variabilité des conditions environnementales liées à la voie d'accumulation (concentration métallique dans l'eau de mer et dans la nourriture, influence de la qualité et quantité de nourriture).

L'influence de la taille du clam sur ses capacités de bioconcentration a été étudiée afin d'établir une gamme de taille permettant de limiter au maximum la variabilité des concentrations métalliques (***Chapitre 1***).

Les bivalves sont exposés dans le milieu marin aux contaminants via différentes voies (dissoutes ou particulaires), et peuvent accumuler les métaux dissouts, les métaux liés aux sédiments et/ou les métaux associés au phytoplancton. **L'influence de la voie d'accumulation** (eau de mer, sédiments, nourriture) des contaminants sur les cinétiques d'accumulation et d'élimination des contaminants dans les espèces cibles a été étudiée en considérant séparément chaque voie d'accumulation (***Chapitres 2, 3 et 4***).

Enfin, l'**effet de la variabilité des conditions environnementales** (concentration métallique dans l'eau de mer et dans la nourriture, influence de la qualité et quantité de nourriture) sur la bioaccumulation des métaux par les espèces sélectionnées a été étudié. En effet, en fonction de leur localisation dans le lagon, les organismes peuvent être exposés à des concentrations métalliques très contrastées. Les gammes de concentrations métalliques rencontrées dans les eaux côtières couvrent plusieurs ordres de grandeurs et atteignent des concentrations extrêmement élevées (e.g. les concentrations en nickel dissout varient de 50 à 1340 ng l^{-1}). De plus, étant donné que les métaux contenus dans les rejets de l'effluent de l'usine Goro-Nickel après le procédé de lixiviation seront principalement présents sous formes dissoutes (Goro-Nickel 2001; Baroudi et al. 2003), une attention particulière a été apportée à la voie dissoute. Ainsi, afin de savoir comment se comportent les organismes vis-à-vis des contaminants (As, Cd, Co, Cr, Mn, Ni et Zn) en fonction de l'intensité de la contamination ambiante, les

cinétiques d'accumulation et d'élimination des éléments ont été déterminées dans les organismes, exposés à des gammes de concentrations en métaux dissouts comparables à celles existants dans le lagon de Nouvelle Calédonie (***Chapitres 4, 5 et 6***).

La voie trophique est aujourd'hui considérée avec une grande attention dans de nombreuses études, et dans ce présent travail, plusieurs expériences ont été menées afin de déterminer l'effet de la variabilité des conditions contaminantes liée à la voie alimentaire sur le comportement bioaccumulateur des deux bivalves *G. tumidum* et *I. isognomon* (***Chapitre 7***). Tout d'abord, l'influence de la concentration en Co à laquelle le phytoplancton a été préalablement exposé (jusqu'à 500 ng l^{-1}) a été déterminée sur l'assimilation et la rétention du Co ingéré avec le phytoplancton dans les bivalves. Puis, comme la disponibilité de la nourriture est variable au cours du temps dans le milieu marin, les bivalves ont été exposés à des conditions de nourriture variables en terme de souches phytoplanctoniques (*Heterocapsa triquetra, Emiliania huxleyi* et *Isochrysis galbana*) et de quantités disponibles (de 5 10^3 à 5 10^4 cell ml^{-1}) afin de déterminer l'influence des aspects qualitatifs et quantitatifs de la nourriture sur l'assimilation et la rétention des contaminants dans les deux bivalves.

Le suivi des cinétiques de transfert des métaux dans les organismes à partir de leur environnement est extrêmement difficile à quantifier (concentrations faibles, variabilité biologique élevée, etc.). Pour surmonter cette difficulté, dans cette première partie, le suivi de la bioaccumulation des métaux a été réalisé en faisant appel aux méthodes de radiotraçage. Les isotopes radioactifs des éléments sélectionnés et les techniques de détection nucléaire qui ont été utilisées sont la spectrométrie gamma pour ^{110m}Ag, ^{73}As, ^{109}Cd, ^{57}Co, ^{51}Cr, ^{54}Mn, ^{65}Zn et la spectrométrie bêta pour le ^{63}Ni. En effet, les radioisotopes ne diffèrent de l'élément stable correspondant que par leur nombre de neutrons et possèdent donc les mêmes propriétés chimiques. Par ailleurs, les techniques de détection nucléaire sont extrêmement sensibles et permettent d'étudier des concentrations contaminantes représentatives de celles réellement rencontrées dans le milieu naturel (y compris les plus faibles) sans perturber les propriétés physico-chimiques du milieu expérimental. La spectrométrie γ est par ailleurs une méthode de mesure non destructive qui permet de suivre l'intégralité de la cinétique d'accumulation et d'élimination d'un radiotraceur dans chacun des individus expérimentés (ce qui permet de réduire la variabilité biologique de façon considérable). La spectrométrie β est au contraire une technique de mesure destructive nécessitant la digestion des échantillons avant analyse (ce qui augmente considérablement le nombre d'organismes nécessaire lors d'une

expérience). Pour cette raison, les métaux ayant des traceurs radioactifs émetteurs γ (^{73}As, ^{110m}Ag, ^{109}Cd, ^{57}Co, ^{51}Cr, ^{54}Mn, ^{65}Zn) seront considérés par la suite séparément du ^{63}Ni, émetteur β. Les biocinétiques présentées ci-après sont toutes réalisées sur le même schéma expérimental-type : elles comprennent une phase d'accumulation (2 à 4 semaines) pendant laquelle les organismes sont placés en conditions contaminantes (concentration en radiotraceurs maintenue constante), suivie d'une phase d'élimination pendant laquelle les organismes ne sont alors plus exposés aux radiotraceurs, mais replacés en conditions non contaminantes en circuit ouvert. Les organismes ont été soumis à une contamination métallique multi-élémentaire par l'intermédiaire de l'eau de mer, des sédiments (préalablement radiomarqués), ou de la nourriture (préalablement radiomarquée) en conditions contaminantes faibles (concentration bruit de fond), et à une contamination mono-élémentaire en conditions contaminantes variables (gamme de concentrations).

L'objectif de cette première partie a été de caractériser la valeur bioindicative de l'algue brune *Lobophora variegata*, des huîtres *Isognomon isognomon* et *Malleus regula*, et du clam *Gafrarium tumidum* en conditions contrôlées, de déterminer les facteurs pouvant influencer la bioaccumulation des contaminants dans ces espèces, et d'établir les recommandations nécessaires à l'utilisation fiable de ces organismes en tant que bioindicateurs.

Les résultats de cette première partie sont rapportés sous formes de 7 articles scientifiques.

CHAPITRE 1

Aspects allométriques de la bioconcentration des métaux lourds dans le clam tropical *Gafrarium tumidum*

La contamination métallique est un problème majeur dans le lagon de Nouvelle-Calédonie, principalement en raison des activités minières existant sur l'île. Or, très peu d'informations sont disponibles sur l'écotoxicologie des métaux dans les organismes marins locaux. Le clam *Gafrarium tumidum* a été choisi afin d'évaluer son utilisation en tant qu'espèce bioindicatrice de la contamination métallique du lagon. Plus particulièrement, les relations allométriques entre l'accumulation en métal et la taille du clam ont été déterminées pour 5 métaux présents dans les eaux du lagon de Nouvelle-Calédonie (Cd, Cr, Co, Zn et Ag) à l'aide des techniques extrêmement sensibles de radiotraçages. Les résultats expérimentaux ont montré que les relations allométriques étaient dépendantes de l'élément et du compartiment corporel considérés. En règle générale, les relations allométriques entre le facteur de concentration des métaux et la taille du clam sont plus prononcées dans la coquille que dans les parties molles. Des relations significatives de type puissance inverse ont été observées entre la taille du clam et le FC des clams pour le Cd, le Cr, le Co et le Zn. En revanche, la bioconcentration de l'Ag est corrélée positivement avec la taille. Par rapport aux données disponibles dans la littérature sur l'Ag dans les bivalves, la dernière observation suggère la présence d'un mécanisme spécifique de détoxication (séquestration) qui serait plus efficace dans les individus les plus âgés. De façon générale, les résultats expérimentaux indiquent que l'utilisation du clam *Gafrarium tumidum* comme bioindicateur dans des programmes de biosurveillance nécessite la sélection d'individus avec une classe de taille spécifique afin d'obtenir des informations comparables sur les niveaux de concentration des métaux ambiants. Puisque l'effet de taille est le plus important parmi les petits individus, il est recommandé de choisir des clams avec une largeur de coquilles supérieures à 35 mm.

Allometric relationships in the bioconcentration of heavy metals by the edible tropical clam *Gafrarium tumidum*

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ABSTRACT. Although metal contamination is a problem of major concern in the lagoon of New Caledonia due to intense mining activities conducted on land, very little is known on the metal ecotoxicology of local marine organisms. The clam *Gafrarium tumidum* was investigated to assess its usefulness as a bioindicator species of metal contamination in this lagoon. More particularly, allometric relationships between metal accumulation and clam size were determined for five common metals in New Caledonian lagoon waters (Cd, Cr, Co, Zn and Ag) using a highly sensitive radiotracer technique. Experimental results showed that allometric relationships were dependent on the element and on the body compartment considered. As a rule, allometric relationships of metal concentration factor were more pronounced in shell than in soft parts. Significant relationships with clam size for Cd, Cr, Co and Zn followed inverse power functions. In contrast, the degree of Ag bioaccumulation was positively correlated with size. In view of the literature on Ag in bivalves, the latter observation suggests the occurrence of a specific detoxification mechanism (sequestration) that would be more efficient in old individuals. Overall, the experimental results indicate that the use of *G. tumidum* as a bioindicator in monitoring programmes requires selecting individuals of a specific size range in order to obtain comparable information about ambient metal levels. Since the size effect is greatest among smaller individuals, it is recommended to select clams with a shell width longer than 35 mm.

Keywords: Size effect, metal, uptake, clam, tropical lagoon, New Caledonia

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I. INTRODUCTION

The lagoon of New-Caledonia is the second largest one in the world (Labrosse et al. 2000). It is subjected to increasing urban development and to intensive mining activities, both of which result in important anthropogenic inputs of heavy metals. The mining industry, which is the major economic resource of New Caledonia, mainly concerns Ni but other metals (Cr, Fe, Co, Mn, Cu, Pb, Ag) are or have been exploited as by-products as well. Nowadays, the ore extracted from open-cast mines is partly processed locally in a melting plant. Recent development of more efficient extraction processes based on acidic extraction (viz. lixiviation) is now making extraction from low ore laterite possible; hence, strong mining development is underway (Mihaylov et al. 2000; Goro-Nickel 2001; Dalvi et al. 2004). Several authors are concerned about these new hydrometallurgic processes as they are likely to bring new, additional risks for the environment (Goro-Nickel 2001; Baroudi et al. 2003). Indeed, the acidic solubilization of metals will obviously not be restricted to extracted Ni but will also concern all other ore-contained by-product metals. Therefore there is a non negligible risk that application of lixiviation process will eventually result in increased discharges of dissolved by-product metals into the environment.

Although it is established that mining enhance metal discharges as well as turbidity and sedimentation rates in the New Caledonian lagoon (e.g., Bird et al. 1984; Laganier 1991; Ambatsian et al. 1997), very little information is available regarding levels of metal contamination and possible impact on the local marine ecosystems.

Among the common approaches used to study environmental contamination, the use of bioindicator species proved to be a valuable and informative approach. Bivalves are the most widely used bioindicators in monitoring studies of marine metal contamination (e.g. Mussel Watches; Goldberg et al. 1978; Boyden & Phillips 1981; Goldberg et al. 1983). They are well known to bioconcentrate metals and to provide a time-integrated indication of environmental contamination. Generally, it is recommended that bioindicator organisms should concentrate target contaminants, be sessile or sedentary, widely distributed, available all year long and easy to collect (Moore 1966; Phillips 1976a; Phillips & Segar 1986; Phillips 1990). In addition, biotic and/or abiotic factors that are likely to affect metal levels in the target species (such as temperature, salinity, season, size/age or sex) should be characterized (Cossa et al. 1979; Strong & Luoma 1981; Newman & McIntosh 1991; Wang & Fisher 1997b). Among

these factors, individual size/age is known to be of primary importance (Boyden 1974; Brix & Lyngby 1985; Warnau et al. 1995).

Available data on metal contamination in biota from New Caledonia is scarce. However, a recent study (Breau 2003) screened metal concentrations in a variety of local marine organisms from several parts of the lagoon with supposed contrasted contamination status and showed that a few local species could be considered as potential bioindicators. Among these species, the edible clam *Gafrarium tumidum* appears as a key species because of its abundance, easiness of sampling and wide distribution in the lagoon as well as in other tropical ecosystems (Baron & Clavier 1992a).

In the context of a research project aimed at developing a local biomonitoring programme, the infaunal clam *G. tumidum* was selected as a potential target species. The objective of the present study was to determine the possible relationships between body size of *G. tumidum* and the capacity of the clam to bioconcentrate selected metals known to be by-products of Ni mining (viz. Cd, Cr, Zn, Co and Ag) and, therefore to be able to select the most appropriate individual size range for use in future biomonitoring studies. Though bioaccumulation in marine bivalves occurs via both seawater and feeding pathways, the present study concentrates on the direct uptake from seawater. Indeed, complementary experiments (Hédouin et al. unpublished work) have shown that seawater and food pathways grossly contribute equally to the global bioaccumulation of the selected elements in *G. tumidum*. Therefore, any allometric effect on bioconcentration from seawater would affect the global bioaccumulation in clam individuals.

II. MATERIALS AND METHODS

II.1. COLLECTION AND ACCLIMATION OF ORGANISMS

Gafrarium tumidum individuals (n = 55; shell width from 21.3 to 45.1 mm; whole body wet wt from 3.83 to 35.03 g) were collected in September 2002 at Ouano Beach, on the southwest coast of New Caledonia and transported to MEL premises in Monaco. Prior to experimentation they were acclimated for 2 months to laboratory conditions (open circuit 400 l aquarium; water renewal rate: 40 l hr^{-1}; salinity: 36 p.s.u.; T: 25 ± 0.5°C; light/dark cycle: 12 hrs / 12 hrs) simulating the conditions prevailing in New Caledonian lagoon waters. During acclimation, clams were fed phytoplankton mixture (*Isochrysis galbana, Heterocapsa*

triquetra, Thalassiosira pseudonana, Emiliania huxleyi); recorded mortality was lower than 4 %.

II.2. EXPERIMENTAL RADIOTRACER PROCEDURE

A set of 53 clams was placed for 15 d in a glass aquarium containing 70 l of natural seawater (salinity: 36 p.s.u.; T = 25 ± 0.5°C; pH = 8.0 ± 0.1). The exposure duration was adapted from preliminary experiments of longer duration (Metian 2003), which indicated that *G. tumidum* incorporates the considered metals according to linear uptake kinetics over more than one month. Hence, reaching a steady-state would need a much longer time. Therefore, a 15-d exposure was selected as it allowed reaching easily detectable metal levels in the clam and was representative of the uptake behaviour of the clam more than one month.

Physico-chemical parameters of the seawater (salinity, temperature, pH) were checked twice daily all along the experiment duration. The target metals were introduced into the experimental microcosms as high-specific activity radiotracers purchased from Amersham, UK (^{51}Cr as Na_2CrO_4 in saline solution, $T_{½}$ = 27.7 d; ^{57}Co as $CoCl_2$ in 0.1M HCl, $T_{½}$ = 271.8 d), CERCA, France (^{110m}Ag as $AgNO_3$ in 1M HNO_3, $T_{½}$ = 249.8 d,), and Isotope Product Lab., USA (^{109}Cd as $CdCl_2$ in 0.1M HCl, $T_{½}$ = 426.6 d; ^{65}Zn as $ZnCl_2$ in 0.5M HCl; $T_{½}$ = 243.9 d). Seawater was spiked with low nominal activities of each selected radiotracer: 3 kBq l^{-1} ^{51}Cr, 1 kBq l^{-1} ^{57}Co, 0.7 kBq l^{-1} ^{65}Zn, 3 kBq l^{-1} ^{109}Cd, 0.7 kBq l^{-1} ^{110m}Ag. In terms of stable metal concentration, these additions corresponded to 2.8 10^{-10} g Cr l^{-1}, 5.5 10^{-11} g Co l^{-1}, 8.4 10^{-9} g Zn l^{-1}, 1.5 10^{-10} g Cd l^{-1} and 3 10^{-8} g Ag l^{-1}, which are 1 to 3 orders of magnitude lower than the background concentrations of these metals in open seas (Bruland 1983). No change in pH was detectable after tracer addition. Seawater and spikes were renewed daily for 5 d, then every second day in order to keep exposure activities as constant as possible. Activities of the metal tracers in seawater were checked daily, before and after each seawater renewal, to determine the time-integrated activities (Warnau et al. 1996b). The maximum decrease in seawater radioactivity between two successive seawater renewals was 33 % (^{110m}Ag). For the entire experimental time course, the time-integrated tracer activities in seawater were 2.80 kBq ^{51}Cr l^{-1}, 0.83 kBq ^{57}Co l^{-1}, 0.55 kBq ^{65}Zn l^{-1}, 2.58 kBq ^{109}Cd l^{-1} and 0.56 kBq ^{110m}Ag l^{-1}). During the experiment duration, the clams were allowed to feed briefly (30 min) on phytoplankton *Isochrysis galbana* in clean seawater every second day, before a seawater and spike renewal in order to minimize any possible ingestion of radiotracer through the food.

At the end of the period of exposure, the clams were dissected to separate soft parts from shells. The two body compartments were then weighed and radioanalyzed to measure their respective radiotracer activities.

II.3. RADIOANALYSES

Radioactivity was measured using a high-resolution γ-spectrometer system (Germanium -N or P type- detectors EGNC 33-195-R, Intertechnique) connected to a multichannel analyser (Intergamma, Intertechnique). The radioactivity of samples was determined by comparison with standards of known activities and of appropriate geometry. Measurements were corrected for counting efficiency and radioactive decay. Counting times were adapted to obtain counting rates with propagated errors less than 5 %; typically, counting times ranged from 1 to 2 hrs.

II.4. DATA TREATMENT

Uptake of the five investigated radiotracers was expressed as concentration factors (CF), viz. the ratio between activity of a radiotracer in the whole organism or in a body compartment (Bq g^{-1} wet weight) and the time-integrated activity of this radiotracer in seawater (Bq g^{-1}). CFs were then plotted against individual width (cm) and allometric relationships were tested using simple linear, power, hyperbolic or exponential models (Zar 1996). Constants of the models and their statistics were estimated using routines from STATISTICA®5.1 software. Best fitting regression models were selected according to highest determination coefficient and examination of residuals.

III. RESULTS

Although to different degrees, variations in metal uptake efficiency with body size were observed for all elements examined in shell or in soft parts, as well as in whole clams (reconstructed data) in the considered 2 to 5 cm range for shell width (Table 1). When significant, these allometric relationships were always best described by a power function ($CF = a * size^b$). CF was inversely related to body size for Cr, Cd, Co, and Zn, and positively related for Ag, indicating that smaller (younger) individuals had a higher bioconcentration potential than larger (older) ones for all metals tested except Ag.

Table 1. Parameters and statistics of the power function (CF = a * size^b) best describing the relationships between concentration factor (CF) of five metals and body size (shell width, cm) in whole-body, shells and soft parts of *Gafrarium tumidum* (n = 53).

R^2: determination coefficient; p: probability of the regression parameters (a and b); ASE: asymptotic standard error; n.s.: no significant relationship found ($\alpha = 0.05$).

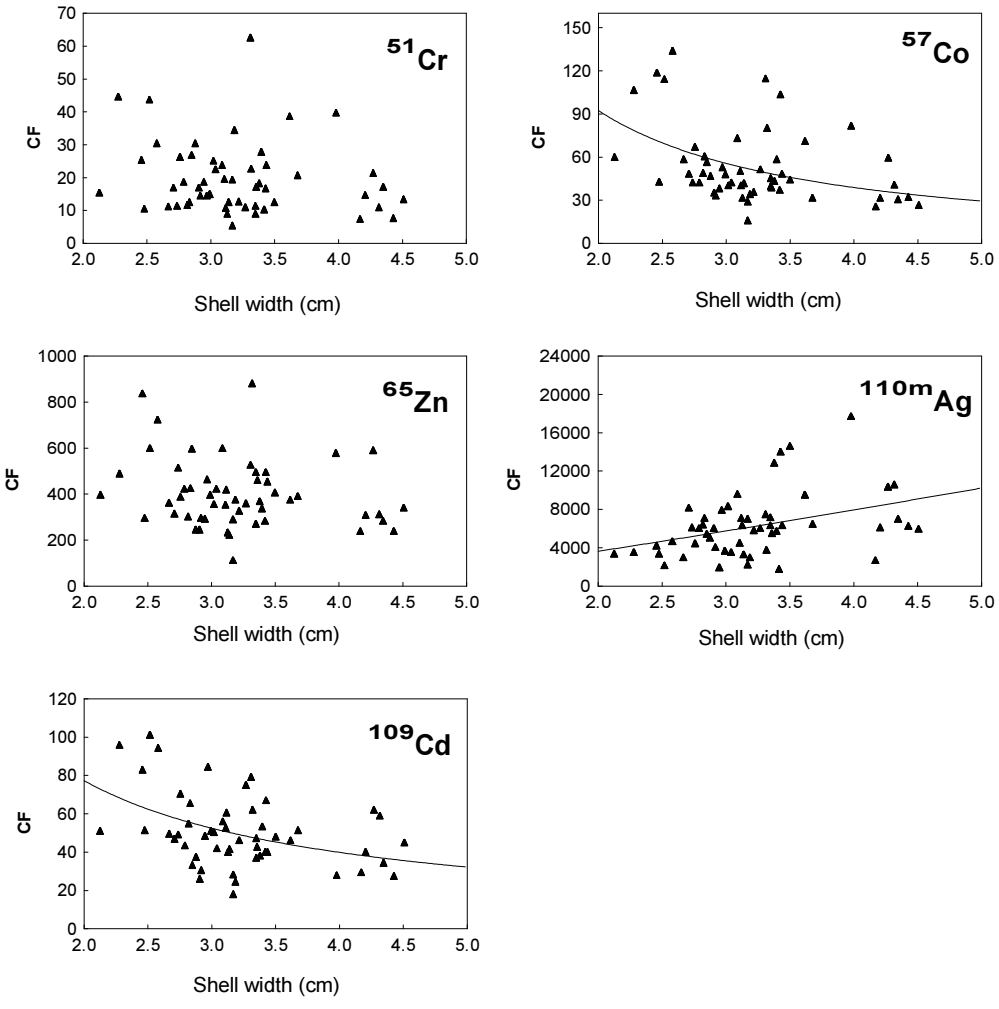
Metals	a	ASE	p(a)	b	ASE	p(b)	R^2
			WHOLE-BODY				
Cr	207	48	<0.0001	-1.52	0.22	<0.0001	0.62
Co	2514	598	<0.0001	-2.23	0.23	<0.0001	0.74
Zn	497	106	<0.0001	-1.2	0.19	<0.0001	0.74
Ag	190	90	0.04	1.08	0.38	0.007	0.18
Cd	21	8	0.008	-0.99	0.32	0.004	0.20
			SOFT PARTS				
Cr							n.s.
Co	219	128	0.09	-1.25	0.54	0.02	0.16
Zn							n.s.
Ag	1662	1169	0.16	1.13	0.56	0.049	0.15
Cd	150	65	0.03	-0.95	0.39	0.02	0.17
			SHELLS				
Cr	227	52	<0.0001	-1.56	0.21	<0.0001	0.62
Co	2762	682	<0.0001	-2.25	0.24	<0.0001	0.73
Zn	519	125	<0.0001	-1.48	0.22	<0.0001	0.57
Ag							n.s.
Cd	8	1	<0.0001	-1.34	0.29	<0.0001	0.29

III.1. SOFT PARTS

Significant allometric relationships were observed for Co, Ag and Cd whereas no significant relationships were found for Cr and Zn, indicating that bioconcentration of these 2 latter elements was not dependent on shell size. Concentration factors were inversely related with size for Co and Cd, whereas Ag presented an opposite behaviour with CFs positively correlated with shell width (Fig. 1). However, even though significant relationships were statistically established for Co, Cd and Ag the quite low determination coefficients ($R^2 < 0.17$) of the power function indicate that the size parameter did exert only a minor influence on the degree of metal uptake by *G. tumidum*.

Figure 1. Relationships between metal concentration factor (CF) in soft parts and shell width (cm) of *G. tumidum.*

Equations best fitting the relationships are given in Table 1.

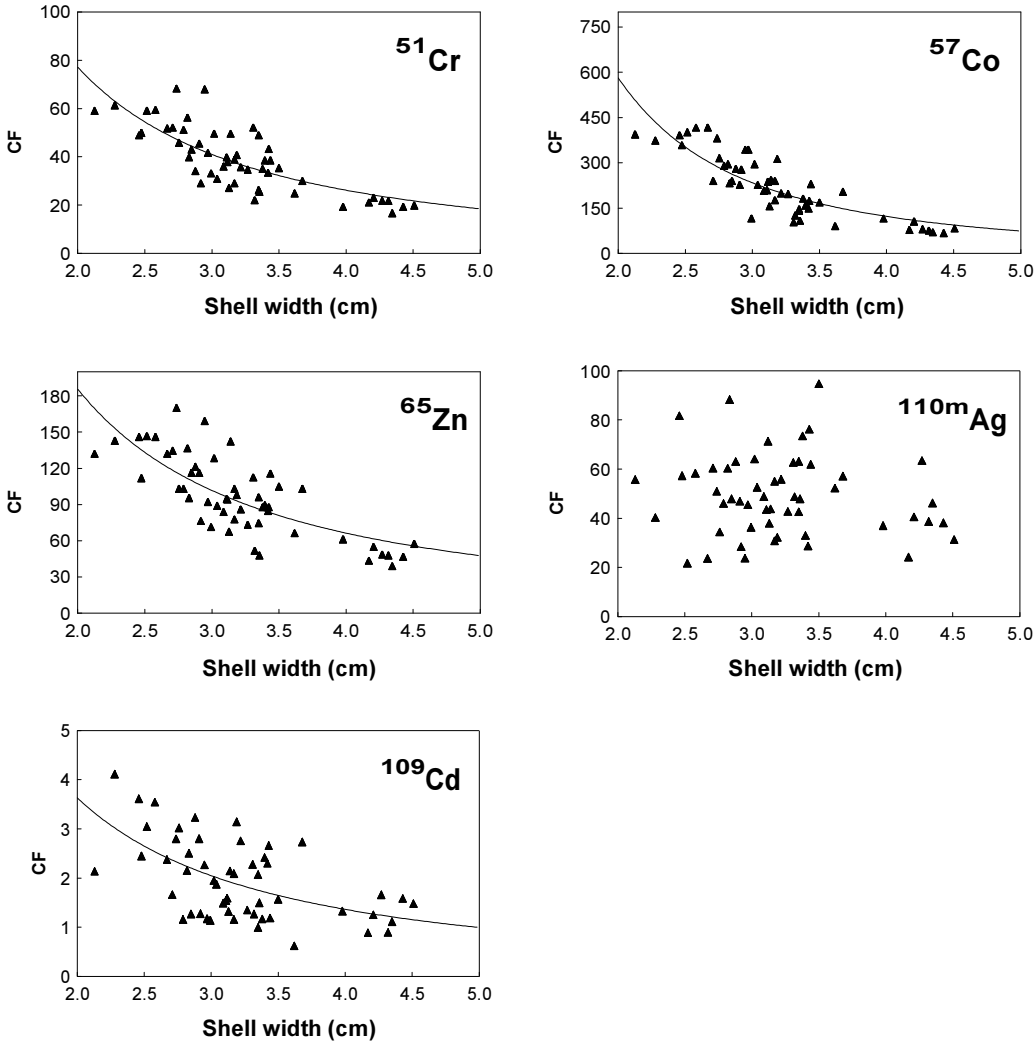


III.2. SHELLS

Cr, Co, Zn and Cd CFs showed a significant inverse power relationship with clam size (R^2 from 0.29 to 0.73) (Fig. 2). In contrast, no significant relationship was found between Ag uptake and size. Cr CFs in shells and soft tissues were roughly of the same order of magnitude whereas CFs of Co, Cd, Zn and Ag were much higher in soft parts than in shells (from a factor 5 to 2 orders of magnitude).

Figure 2. Relationships between metal concentration factor (CF) in shells and shell width (cm) of *G. tumidum.*

Equations best fitting the relationships are given in Table 1.

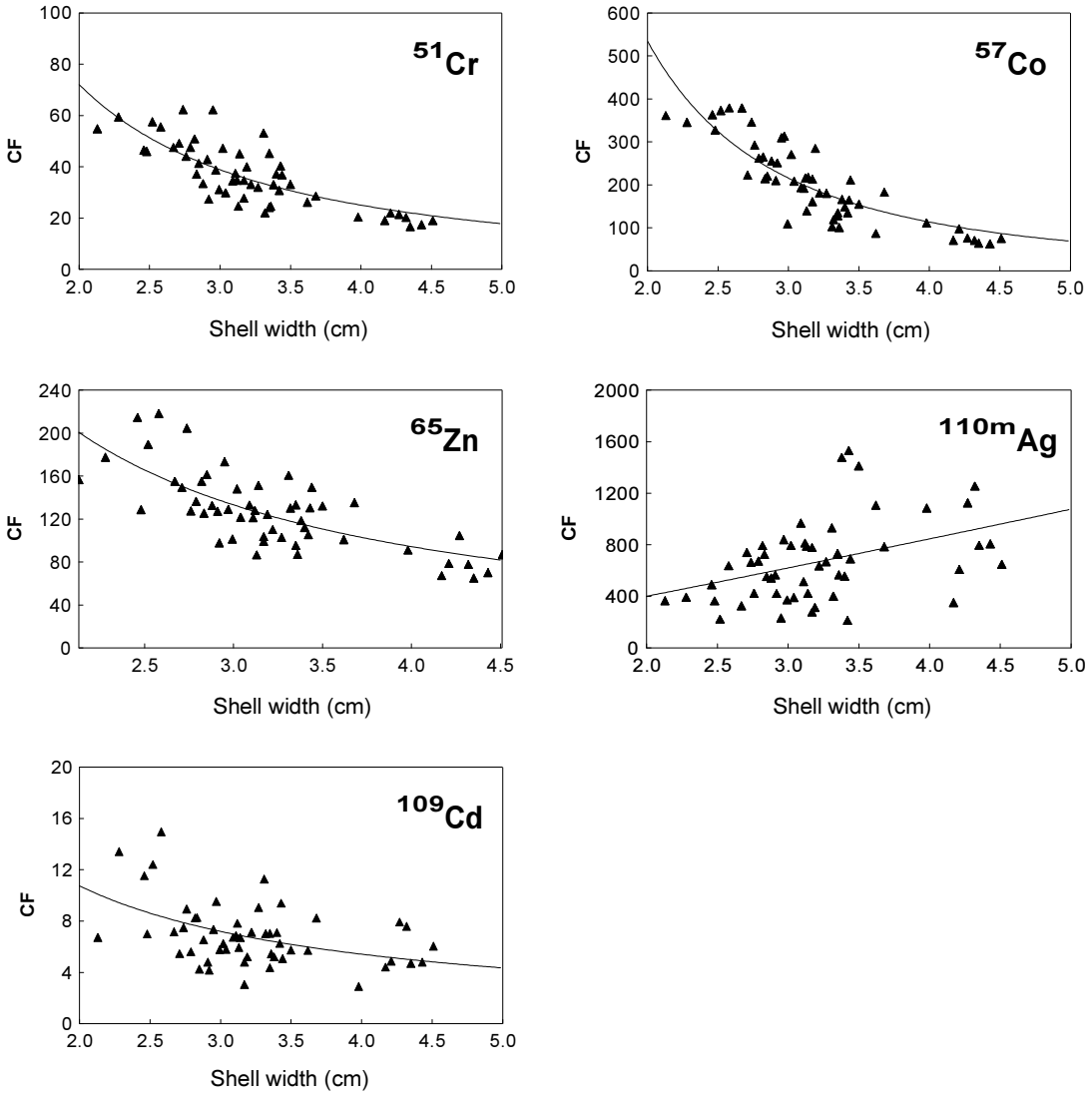


III.3. WHOLE INDIVIDUALS

Significant allometric relationships were found in whole organisms (from reconstructed data) for all the metals (Fig. 3). Relationships between Cr, Co and Zn CFs and size displayed quite high determination coefficients (R^2 = 0.62, 0.74 and 0.74, respectively) whereas Ag and Cd were characterized by R^2 values of 0.18 and 0.20, respectively. As in the case of shells, CFs of all metals except Ag decreased with increasing clam size. Ag was the only element tested for which CF was positively related to shell width.

Figure 3. Relationships between metal concentration factor (CF) in whole organisms and shell width (cm) of *G. tumidum*.

Equations best fitting the relationships are given in Table 1.



IV. DISCUSSION

Body size is a factor that is well-known to affect metal concentrations in organisms (e.g., Strong & Luoma 1981; Beeby 1991; Newman & McIntosh 1991; Warnau et al. 1995); however, the mechanisms underlying these effects of size remain poorly understood. The two explanations that are generally given are (1) the decreasing surface/volume ratio of an organism with increasing body size, and (2) decreasing metabolic activity in larger (older) organisms (Boyden 1974, 1977; Swaileh & Adelung 1995). Both parameters would tend to result in decreasing metal uptake with increasing individual size. However, available literature clearly shows that a single trend does not exist. In bivalves for instance, whole body concentrations of metals may either decrease, increase or remain constant with increasing body size depending both on the metal and the species considered (Boyden 1974, 1977; Cossa 1980; Brix & Lyngby 1985).

The present work investigated the effect of body size (age) on metal bioconcentration in the clam *Gafrarium tumidum*. This approach is somewhat different from the one usually carried out to study size effect. Indeed, allometric variations are most generally determined by investigating relationships between metal concentrations and body size (Swaileh & Adelung 1995; Cubadda et al. 2001; Saavedra et al. 2004), whereas here we investigated the relationships between relative metal bioconcentration (as concentration factor, CF) and size. Thus our radiotracer study has an intrinsic mechanistic objective since it directly determines the possible variation in bioconcentration potential according to age of individuals (Wang & Fisher 1997b; Lee et al. 1998; Wang & Dei 1999).

Although we observed the three different possible trends (increasing, decreasing and non significant relationships) between clam size and the degree of bioconcentration of the five metals tested (Cr, Co, Cd, Zn, Ag), the most common allometric relationship was a decreasing metal uptake with increasing size of the clams. In fact, significant inverse power relationships between CF and body size were found for all metals except Ag. Such an inverse trend suggests that young organisms generally have a higher bioconcentration potential than the older ones.

The inverse relationships between CFs of Cd and Co and size that were noted in clam soft parts are in accordance with observations reported from previous works with the mussels *Mytilus edulis* (Wang & Fisher 1997b) and *Septifer virgatus* (Wang & Dei 1999). This

decreasing trend could be explained by the decreasing surface/volume ratio of tissues with increasing body size. However, some biological processes are likely to take part in the observed allometric relationships as well. Indeed, previous studies have shown that the metabolic rate decreases with age (e.g., Hamburger et al. 1983) and/or that metal behaviour in the organism varies according to the different life stages (e.g., Williamson 1980). Similarly, physiological parameters that characterize exchanges between the organism and its environment such as filtration rate, O_2 consumption, or respiration rate, have been shown to vary as a function of tissue weight, with higher values in younger organisms due to the difference of gill surface area and growth rate (Hamburger et al. 1983; Sukhotin et al. 2003). Furthermore, similar allometric coefficients have been computed for e.g. Cr uptake and filtration activity, suggesting that both processes could be controlled by the same mechanism(s) (Wang & Dei 1999).

For Zn and Cd, Wang & Dei (1999) found uptake coefficients that were higher than those of filtration activity, which supported the hypothesis that uptake of these metals was controlled by other mechanisms such as the binding of metals with bioavailable ligands (Wang & Fisher 1997b; Lee et al. 1998; Wang & Dei 1999). In the case of Cd, the higher degree of bioconcentration in the younger clams observed here could thus also be due partly to the presence of metallothioneins (MTs). These proteins of low molecular weight are characterized by their elevated content in cysteins (20-30 %). MTs are able to complex metals such as Hg, Ag, Cu, Zn and Cd, (e.g., Bebianno et al. 1993; Perceval et al. 2002) and played an important role in detoxification processes. Although there is no direct evidence published on the occurrence of MTs in *G. tumidum*, numerous works have been reporting metallothioneins in a wide diversity of molluscs, including clam species (e.g., Bebianno & Langston 1992; Bebianno et al. 1993; Gnassia-Barelli et al. 1995; Geret et al. 2002; Bebianno et al. 2003). In addition it has been documented that metallothionein content is size-dependent; viz. MTs may be more abundant in either small (Bordin et al. 1997; Mouneyrac et al. 1998) or large (Serafim et al. 2002; Bebianno et al. 2003) individuals. Therefore, a higher metallothionein content in young clams could be partly responsible for their higher bioconcentration of Cd compared to that in larger individuals.

CF of Cr and Zn did not display any significant allometric relationships in the soft parts of the clams, indicating that concentration of these elements from seawater would remain similar throughout the lifespan of the clam. Similar results have been reported for Zn uptake in the blue mussel *Mytilus edulis* (Williamson 1980; Strong & Luoma 1981; Brix & Lyngby 1985).

The constant degree of uptake of Zn and Cr over the entire size range of *G. tumidum* is very important regarding the use of the clam as a bioindicator species in the framework of monitoring programmes. Indeed, this suggests that Zn and Cr concentrations measured in soft tissues would be directly comparable among *G. tumidum* individuals, regardless of their size.

In contrast to the other metals examined, Ag acted as a cumulative contaminant: it was concentrated in soft parts and in whole clams to a higher degree in larger individuals. Although the observed allometric relationships were relatively weak ($R^2 < 0.18$), this finding is somewhat surprising since Ag is a non-essential element and one of the most toxic metals to invertebrates (Bryan 1984). However, some bivalves accumulate Ag up to very high levels by trapping it as Ag_2S which is an insoluble, non-toxic, and stable compound (Martoja et al. 1988; Martoja et al. 1989; Berthet et al. 1990; Berthet et al. 1992). In fact a positive correlation between Ag concentrations in soft parts and body size has also been observed for the clam *Macoma bathica* (Strong & Luoma 1981). Thus in *G. tumidum*, the occurrence of a similar detoxification/sequestration system that would be slightly more efficient in older clams could explain the observed increasing bioconcentration efficiency with age.

Regarding *G. tumidum* shells, the allometric trends generally observed were a decrease in CF with increasing shell width, a relationship which is in agreement with previous reports for the behaviour of Cr, Cd, Hg and Zn in shell of the blue mussel *M. edulis trossulus* (Brix & Lyngby 1985). Bivalve shells are composed primarily of calcium carbonate secreted by mantle tissues that take up Ca^{2+} ions from seawater and incorporate it in the shell. However, the Ca^{2+} ions may be replaced by other metal ions with similar radius, and then incorporated into the inner layers of the shells (Cravo et al. 2002). This biologically-mediated uptake of metals into shell occurs simultaneously with metal adsorption onto the outer shell surface (because of the permanent contact of shells with ambient seawater) (Bourgoin 1990; Puente et al. 1996). However, in our experimental study the relative short duration of the experiment (15 d) limited the formation of new shell and biological incorporation of metals into the shell. Thus variations in metal uptake observed in the shell was most probably a result of adsorption processes which follow the general trend of decreasing surface/volume ratio with increasing size of the organism (White & Rainbow 1987).

It has to be noted that steady-state equilibrium was not reached in the clams over the experimental time course; hence, one could argue that in the longer term distributions across size range could be different. Although this cannot be totally discarded, our preliminary longer-term experiments indicated that *G. tumidum* concentrates the investigated elements

linearly over more than one month (Metian 2003). Therefore, the differences in size-related bioconcentration efficiency observed here will be maintained at least for a few months. Furthermore, conclusions from a preliminary *in situ* study on allometric relationships of Cr, Co and Zn in *G. tumidum* individuals supposedly in equilibrium with ambient environmental conditions (Breau 2003), strongly converged with the experimental trends reported here, hence supporting the long-term validity of our observations.

In conclusion, our findings emphasize the need to consider body size as a limiting factor when using the clam *G. tumidum* as a bioindicator species in tropical waters. In particular, our results show that the capacity of incorporating metals generally varies during the lifespan of the clam: smaller (younger) individuals tend to display higher metal uptake than larger (older) ones. The inverse power relationships generally observed between CF and body size imply that in smaller clams, slight differences in body size could result in large variations in tissue metal concentration. Consequently, if the clam is used in biomonitoring studies, individuals with a shell width longer than 35 mm should be selected to minimize the body size effect on metal concentrations in tissues. Even if stable metal concentrations measured in the large clams are lower than those in small clams (due to the lower degree of uptake by the former), these levels are sufficient to ensure precise and accurate analyses with high reproducibility (Breau 2003).

Ag uptake showed allometric relationships opposite to those found for the other elements examined; i.e. the uptake of Ag increased with increasing clam size. This is probably due to the occurrence of a detoxification process leading in Ag_2S formation and deposition in the clam tissues. If this hypothesis is verified, *G. tumidum* could be used as a bioindicator of chronic Ag contamination over the very long term, since Ag concentrations in soft tissues would probably reflect the metal sequestrated during the entire lifespan of the individuals. Nevertheless, in order to refine the use of *G. tumidum* as a bioindicator species, additional information is still needed, in particular on the rates of uptake and loss of these metals and their distribution in clam tissues. From the results presented here, it is recommended to obtain this information using large *G. tumidum* individuals with > 35 mm shell width.

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CHAPITRE 2

Discrimination des voies d'accumulation des métaux lourds dans des huîtres marines tropicales à l'aide des techniques de radiotraçage

Les cinétiques d'accumulation et d'élimination de 5 radiotraceurs de métaux lourds (^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd, ^{110m}Ag) ont été déterminées dans les huîtres tropicales *Isognomon isognomon* et *Malleus regula* exposées aux métaux par l'intermédiaire de différentes sources de contamination : l'eau de mer, les sédiments et la nourriture. L'accumulation dans les huîtres exposées aux contaminants dissouts est décrite par une cinétique linéaire pour l'Ag, le Cd et le Zn, et une saturation exponentielle pour le Co et le Cr. Dans tous les cas, les métaux dissouts sont bioconcentrés efficacement, principalement sur les coquilles pour le Cr et le Co, et dans les tissus mous pour le Cd, le Zn et l'Ag. L'accumulation des métaux liés aux sédiments dans les deux huîtres est décrite par une cinétique à saturation caractérisée par des facteurs de transfert à l'équilibre très faibles ($TF_{SS} < 0.3$), indiquant une faible biodisponibilité des métaux liés aux sédiments (3 à 5 ordres de grandeur plus faibles que les métaux dissouts). Après la période d'exposition aux métaux via la voie dissoute et sédimentaire, les huîtres ont été placées en condition non contaminante, et la cinétique d'élimination des métaux incorporés a été suivie. Les cinétiques d'élimination dans les deux huîtres sont décrites par un modèle à double exponentielle; le Cd et le Zn sont retenus efficacement dans les deux huîtres (temps de demie vie biologique, $T_{b½} > 2$ mois). Les deux huîtres présentent le même comportement vis-à-vis des métaux, à l'exception de l'Ag et dans une moindre mesure du Cd. L'Ag est incorporé et retenu plus efficacement dans les tissus d'*Isognomon isognomon* que de *Malleus regula*, suggérant des mécanismes de détoxication et de stockage de l'Ag différents dans les deux espèces. De même, le Cd dissout est généralement bioconcentré et retenu de manière plus efficace dans *I. isognomon*. Enfin, les expériences d'alimentation ont montré que les métaux ingérés avec la nourriture (phytoplancton) étaient efficacement assimilés (l'efficacité d'assimilation, EA varie de 34 à 77 %) et fortement retenus dans les tissus des

deux huîtres ($T_{b½}$ de 8 à 20 jours). En conclusion, les deux huîtres *I. Isognomon* et *M. regula* pourraient être d'excellentes espèces bioindicatrices pour surveiller le degré de contamination des écosystèmes tropicaux.

Delineation of heavy metal uptake pathways (seawater, food and sediment) in tropical marine oysters using radiotracers

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To be submitted

ABSTRACT. The uptake and loss kinetics of 5 heavy metal radiotracers (^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd, ^{110m}Ag) were studied in the tropical oysters *Isognomon isognomon* and *Malleus regula* exposed via different sources of contamination: seawater, sediment and food. The whole-body uptake in oysters exposed via seawater followed linear kinetics for Ag, Cd and Zn, and exponential saturation for Co and Cr. In all cases, metals dissolved in seawater were efficiently bioconcentrated, mainly on shells for Cr and Co, and in soft tissues for Cd, Zn and Ag. Uptake from sediments displayed saturation kinetics characterized by low transfer factors at steady state ($TF_{ss} < 0.3$), indicating a low bioavailability of sediment-bound metals (3 to 5 orders of magnitude lower than waterborne metals). After seawater and sediment uptake experiments, loss kinetics of the incorporated metal radiotracers were followed in oysters replaced in non-contaminated conditions. Whole-body loss kinetics were best described by a double exponential equation; Cd and Zn were retained for the longest period (biological half-life, $T_{b½} > 2$ mo). Interestingly, the two oyster species displayed the same behaviour towards all metals except Ag and to a lesser extend Cd. Ag was much more efficiently incorporated and retained in tissues of *I. isognomom* than in *M. regula*, suggesting different mechanisms of metal detoxification/storage in the two species. Dissolved Cd was generally more bioconcentrated and better retained in *I. isognomon* than in *M. regula*. Finally, feeding experiments showed that metals ingested with food (phytoplankton) were efficiently assimilated (assimilation efficiency -AE- ranging from 34 to 77 %) and strongly retained in

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oyster tissues ($T_{b½}$ ranging from 20 d to ∞). It is concluded that *I. isognomon* and *M. regula* could be valuable organisms to be used as bioindicators to survey and monitor metal contamination in tropical ecosystems.

Keywords: Bioaccumulation, *Isognomon isognomon, Malleus regula*, Metal, Kinetics

I. INTRODUCTION

Environmental stress of coastal ecosystems is a worldwide issue that receives growing concern and call for efficient monitoring actions. Coral reef lagoon ecosystems, known for their extreme biodiversity respond to environmental stress in a complex way. However background information in tropical ecosystems is often weak and sparse, especially compared to those available for temperate systems (Fichez et al. 2005). The lagoon of New-Caledonia is one of the largest worldwide (Labrosse et al. 2000). Despite its importance as major natural, recreational and fisheries resources, it is subject to increasing anthropogenic pressures, mainly related to urban development and open-cast mining activities. Mining industries, primarily based on the extraction of Ni and to a much lesser extend of other metals (Co, Cr, Cu, Fe, Mn and Pb), are the major economic resource of New Caledonia. Although it is well known that mining results in high metal discharges and increase in turbidity and sedimentation in the New Caledonian lagoon (Laganier 1991; Ambatsian et al. 1997), very little information is available regarding levels of contamination and possible impairments of the local marine ecosystems. In addition, recent development of more efficient extraction processes based on acidic extraction (viz. lixiviation) is now making extraction from low ore laterite possible and further mining development is underway (Goro-Nickel 2001). These developments will result in increased discharges of metals to the environment, mainly as by-product metals (e.g. Co, Cr, Mn) which behaviour and impact need to be accounted for.

Among the common approaches used to study environmental contamination, bioindicators have been identified as a powerful source of information. Bivalves certainly are the most widely used bioindicators for monitoring marine heavy metal pollution in temperate regions (e.g. Mussel Watches; Goldberg et al. 1983). Some efforts were devoted to the extension of the Mussel Watch to tropical and sub-tropical regions (e.g., UNU 1994), using bivalves such as *Saccostrea* spp., *Crassostrea* spp. and *Perna* spp. as bioindicators (Phillips 1985; Rainbow 1993a). However, none of the latter species is present in sufficient abundance along the New Caledonia coasts to be considered as a useful candidate to monitor local contamination. A recent study screened metal levels in a variety of marine organisms from several parts of the New Caledonia lagoon (Breau 2003). This study showed that a few local species could be considered as potential bioindicators and identified the oysters *Isognomon isognomon* and *Malleus regula* as two of the most promising candidates.

In order to further investigate the potential of these two oysters as bioindicators, the present study used radiotracer techniques to assess bioaccumulation efficiency and kinetics in *I. isognomon* and *M. regula* exposed to 5 selected metals (Cd, Cr, Zn, Co, Ag) via seawater, food or sediment.

II. MATERIALS AND METHODS

II.1. SAMPLE

Oysters were collected by SCUBA diving in September 2002 in Maa Bay, Nouméa, New Caledonia. They were then shipped to IAEA-MEL premises in Monaco, where they were acclimated to laboratory conditions (open circuit, 3000 l aquarium, flux: 300 l h^{-1}; temperature T°: 25 ± 0.5°C; salinity: 36 p.s.u.; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) for two months prior to experimentations. During this period, oysters were fed daily a mixed algal diet (*Isochrysis galbana, Heterocapsa triquetra, Thalassiosira pseudonana, Emiliania huxleyi*).

II.2. RADIOTRACERS AND COUNTING

Uptake and loss kinetics of 5 metals (Cd, Cr, Zn, Co, Ag) in oysters were determined using high specific activity radiotracers purchased from Amersham, UK (^{51}Cr as Na_2CrO_4, $T_{½}$ = 27.7 d; ^{57}Co as $CoCl_2$, $T_{½}$ = 271.8 d), CERCA, France (^{110m}Ag as $AgNO_3$, $T_{½}$ = 249.8 d,), and Isotope Product Lab., USA (^{109}Cd as $CdCl_2$, $T_{½}$ = 426.6 d, ^{65}Zn as $ZnCl_2$, $T_{½}$ = 243.9 d). The tracers were counted using a high-resolution γ-spectrometer composed of three Germanium - N or P type- detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser (Intergamma, Intertechnique). The radioactivity was determined by comparison with standards of known activity and of appropriate geometry. Measurements were corrected for counting efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error less than 5 %.

II.3. EXPERIMENTS

II.3.1. Exposure via seawater

Ten specimens of *Isognomon isognomon* and *Malleus regula* were placed in a 70-l glass aquarium (T°: 25 ± 0.5°C; salinity: 36 p.s.u.; pH: 8.0 ± 0.1) and exposed for 28 d to the 5

radiotracers dissolved in seawater. Nominal activity of each radiotracer was: ^{51}Cr (3 kBq l^{-1}), ^{57}Co (1 kBq l^{-1}), ^{65}Zn (0.5 kBq l^{-1}), ^{109}Cd (3 kBq l^{-1}) and ^{110m}Ag (0.5 kBq l^{-1}). In terms of stable metal concentration, these additions corresponded to Cr (0.3 ng l^{-1}), Co (55 pg l^{-1}), Zn (6 ng l^{-1}), Cd (0.15 ng l^{-1}) and Ag (21 ng l^{-1}), which were 1 to 3 orders of magnitude lower than the natural concentrations of metals in seawater (Bruland 1983). No change in pH was detectable after tracer addition. Spiked seawater was renewed daily during the first 15 d, then every second day in order to keep activities in seawater as constant as possible. Activity of the radiotracers in seawater were checked daily, and before and after each spike renewal, yielding time-integrated activities of ^{51}Cr (2.9 ± 0.2 kBq l^{-1}), ^{57}Co (0.8 ± 0.2 kBq l^{-1}), ^{65}Zn (0.5 ± 0.1 kBq l^{-1}), ^{109}Cd (2.7 ± 0.8 kBq l^{-1}) and ^{110m}Ag (0.4 ± 0.1 kBq l^{-1}).

Oysters were collected at different time intervals and were whole-body radio-counted. At the end of the 28-d exposure period, 6 oysters (n = 3 per species) were sacrificed and dissected. The visceral mass, remaining soft parts and shells were separated, weighed and radio-analysed in order to assess the metal body distribution.

The 14 remaining oysters (n = 7 per species) were then placed in non contaminating conditions (flowing natural seawater, flux: 50 l hr^{-1}, T°: 25 ± 0.5°C; salinity: 36 p.s.u.; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) for 59 d in order to follow the loss of radiotracers from the oyster tissues. Three individuals of both species were collected at the end of the depuration period and dissected to separate whole soft parts from shells and assess metal distribution between these two compartments.

II.3.2. Exposure via the food

Phytoplankton (*Isochrysis galbana)* was exposed for 6 d to the 5 radiotracers, using similar activities as those used for seawater exposure experiment of oysters. At the end of the exposure period, phytoplankton was centrifuged at 2500 × g for 20 min in a Sorvall RC28S ultracentrifuge, then resuspended in clean seawater and the cell density counted. The radioactivity of the labelled *I. galbana* was γ-counted before and after cellular centrifugation.

Twelve oysters (n = 3 *I. isognomon*, n = 9 *M. regula*) were placed in a 70-l glass aquarium (close circuit aquarium constantly aerated, T°: 25 ± 0.5°C; salinity: 36 p.s.u.; pH: 8.0 ± 0.1) and fed radiolabelled *I. galbana* for 2 hrs (10^4 cells ml^{-1}). Empty shells were placed in the aquarium as control for tracer recycling in seawater. The control shells were regularly counted but no activity was detected, indicating no detectable recycling of dietary tracer. After the feeding period (2 hrs), all oysters were γ-counted and flowing seawater conditions were

restored (flux: 50 l hr^{-1}, T°: 25 ± 0.5°C; salinity: 36 p.s.u.; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). Individuals were then γ-counted at different time intervals to follow the loss kinetics of radiotracers in whole-body oysters. Six individuals (n = 3 per species) were collected at the end of the 72-d depuration period and dissected to determine the metal distribution among the different body compartments (gills, mantle, muscle, visceral mass, palps).

II.3.3. Exposure via the sediments

Sediments (3 kg), collected in Sainte-Marie Bay, New Caledonia, were placed for 7d in 3 l of seawater and daily spiked with the 5 radiotracers ^{51}Cr (3 kBq l^{-1}), ^{57}Co (1 kBq l^{-1}), ^{65}Zn (0.5 kBq l^{-1}), ^{109}Cd (3 kBq l^{-1}) and ^{110m}Ag (0.5 kBq l^{-1}). Sediments were then placed in a 70-l aquarium to form a continuous layer of 2 cm height. Weakly bound metals were allowed to leach overnight under flowing seawater. The oysters (n = 10 per species) were then placed in the aquarium (open circuit, flux: 50 l hr^{-1}, T°: 25 ± 0.5°C; salinity: 36 p.s.u.; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) for 35 d. Individuals were regularly radioanalysed during the experiment period (35 d). At the end of that uptake period, 6 oysters (n = 3 per species) were collected, dissected (visceral mass, remaining soft parts and shells), weighed and counted in order to determine the distribution of radiotracer contents among the different body compartments. Remaining individuals were transferred for 49 d to a new aquarium containing clean flowing seawater. At the end of the loss period, 6 oysters (n = 3 per species) were collected and dissected to determine body distribution of the tracers.

II.4. DATA ANALYSES

Uptake of the five investigated radiotracers were expressed in terms of concentration factor (CF, viz. ratio between whole body activity -Bq g^{-1} wet wt- and time-integrated activity of radiotracers in seawater -Bq g^{-1}-). Radiotracer uptake kinetics were described using either a simple linear regression model (eq.1) (linearity was tested by a linearity test for regression with replication, Zar 1996) or a simple first-order exponential kinetic model (eq.2) when observed kinetics tended to reach a steady state:

$CF_t = k_u\, t$ (eq.1)

$CF_t = CF_{ss}\,(1-e^{-k_e\, t})$ (eq.2)

where CF_t and CF_{ss} are the concentration factor at time t (d) and at steady state, respectively;

and k_u and k_e are the uptake and loss rate constant (d^{-1}), respectively (Whicker & Schultz 1982).

Loss of radiotracers was expressed using the percentage of remaining radioactivity (radioactivity at time t divided by the initial radioactivity measured in organisms at the beginning of the decontamination period). The loss kinetics of each radiotracer were fitted by simple linear regression both with and without semi-log transformation of the data. Linearity was tested by a linearity test for regression with replications. If the linearity test was significant when performed on loss kinetics after semi-log transformation, the results were described by a single-component exponential loss model (eq.3):

$$A_t = A_0 e^{-k_e t} \quad \text{(eq.3)}$$

where k_e is the depuration rate constant (d^{-1}); A_t and A_0 are remaining activities (%) at time t (d) and t = 0, respectively.

When the linearity test indicated that retention kinetics could not be described accurately by a single linear regression either with or without semi-log transformation, kinetics were fitted by a 2-component exponential equation. The equation of the model describing these kinetics is a double-component exponential loss model (eq.4):

$$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t} \quad \text{(eq.4)}$$

where k is the depuration rate constant (d^{-1}), and A_t and A_0 are remaining activities (%) at time t (d) and t = 0, respectively ('s' and 'l' subscript for the short-lived and long-lived component).

For each component (short- and long- lived), a biological half-life may be calculated ($T_{b½s}$ and $T_{b½l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b½} = \ln 2/k_e$.

Fitting of the equations and determination of the constants and their statistics were estimated by iterative adjustments of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica software 5.2.1. Differences between estimated kinetic parameters (k_u, k_e, A_{0l}) in both oysters were tested by a Z-Test (Zar 1996), using the standard deviation which was obtained by multiplying asymptotic standard error –ASE- by the square root of the number of individuals. The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

All metals examined, except Ag and to a lesser extend Cd, behaved very similarly or identically in both oyster species regardless the exposure pathways and the uptake/loss phase. Except when specified in the text, kinetic parameters showed no significant difference between the two oyster species ($p > 0.05$).

III.1. SEAWATER EXPERIMENT

Whole-body uptake kinetics of ^{65}Zn, ^{109}Cd, and ^{110m}Ag in the two oysters were best fitted using simple linear regressions ($p < 0.0001$, $R^2 > 0.85$) (Table 1, Fig. 1). Uptake rate constants (k_u) in *I. isognomon* and *M. regula* were similar for ^{65}Zn (mean ± ASE = 103.2 ± 2.4 d^{-1} and 98.0 ± 3.2 d^{-1}, respectively) and significantly different for ^{109}Cd and ^{110m}Ag ($p < 0.0001$), *I. isognomon* showing faster uptake of ^{109}Cd and ^{110m}Ag (k_u = 47.2 ± 1.1 d^{-1} and 121.2 ± 3.0 d^{-1}) than *M. regula* (k_u = 35.6 ± 0.75 $d^{-1}$and 28.2 ± 1.2 d^{-1}). After 28 d of exposure, whole-body ^{110m}Ag concentration factors (CF) were also much higher in *I. isognomon* than in *M. regula* (e.g. $CF_{28d\ in\ toto}$ = 3,244 ± 1,158 vs 783 ± 433) (Table 2).

Regarding ^{51}Cr and ^{57}Co, whole body uptake kinetics were best described by a saturation model. Estimated values of concentration factors at steady state (CF_{ss}) indicated that oysters accumulated about 4 times more ^{57}Co than ^{51}Cr, (CF_{ss} = 976.6 ± 19.3 vs 229.1 ± 5.7 in *I. isognomon* and 1136.4 ± 24.6 vs 206.6 ± 6.6 in *M. regula*) (Table 1). After 28 d of exposure, metal CF calculated in the different body compartments could be ranked in the following order of bioavailability: ^{65}Zn > ^{109}Cd > ^{57}Co > ^{51}Cr (Table 2). ^{51}Cr and ^{57}Co were mainly concentrated onto shells whereas ^{65}Zn and ^{109}Cd were mostly accumulated in visceral mass and in remaining soft parts. In the case of ^{110m}Ag, *I. isognomon* displayed the highest CF in all body compartments

Metal distribution between soft tissues and shells was similar in the two oysters except for ^{110m}Ag and ^{109}Cd. Most of ^{51}Cr and ^{57}Co (97 - 98 %) was associated with the shells, whereas 76-77 % of ^{65}Zn was found in soft tissues. In the case of ^{109}Cd and ^{110m}Ag, the highest proportion of metals was present in soft tissues for *I. isognomon* (55 %) and in shell for *M. regula* (65 and 83 %, respectively). Among soft parts, the remaining soft parts accounted for the main fraction of the total body burden (> 55 %) for all elements tested.

At the end of exposure time, non-contaminated conditions were restored and whole-body loss kinetics of metals were followed for 59 d (Fig. 1). Whole-body loss kinetics were best described by a double exponential model, except for ^{51}Cr, which was best described using a single exponential model (Table 1). The short-lived component of ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag concerned a minor proportion of the total radioactivity (A_{0s} < 24 %), and was characterized by short $T_{b½}$ values ranging from 0.3 d (^{109}Cd) to 2.6 d (^{65}Zn). The long-lived component (A_{0l}) represented the loss of the major proportion of accumulated metals (A_{0l} > 75 %) and displayed $T_{b½}$ ranging from 74 d to the infinite (k_e of ^{65}Zn in both oysters and ^{110m}Ag and ^{109}Cd in *I. isognomon* were not significantly different from 0).

Comparison of the body distribution of radiotracers at the end of uptake and loss period indicated that the distribution remained relatively constant for all metals except for ^{110m}Ag. In *I. isognomon*, ^{110m}Ag content in soft parts increased from 55 % at the end of the uptake period to 87 % at the end of the loss period.

Figure 1. Uptake and loss kinetics of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in the oysters *Isognomon isognomon* and *Malleus regula* exposed for 28 d to 5 radiotracers via seawater (Concentration Factors -CF-; mean ± SD; n = 10 per species), and then maintained for 59 d in non contaminated seawater (Remaining activity -%-; mean ± SD; n = 7 per species).

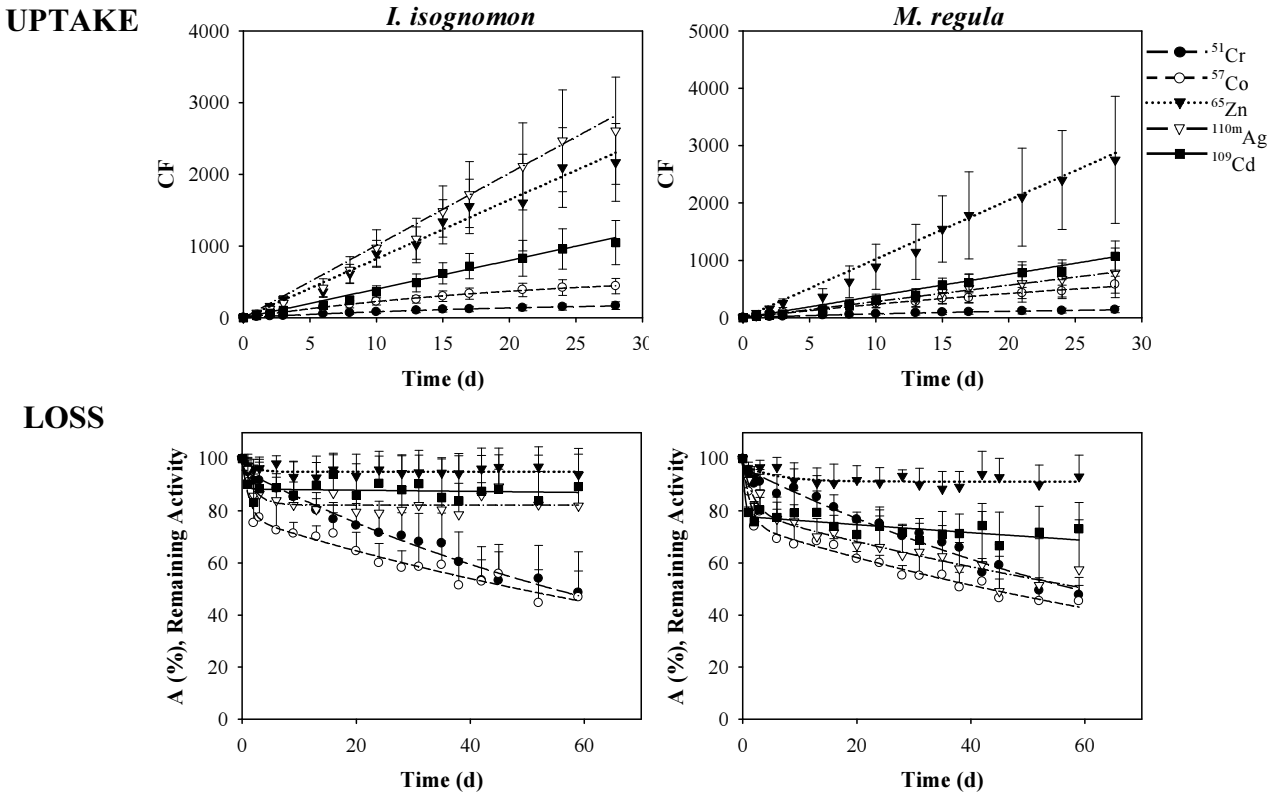

Table 1. Estimated uptake kinetic parameters (A) and loss kinetic parameters (B) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in whole oysters, *Isognomon isognomon* and *Malleus regula,* exposed for 28 d to the radiotracers in seawater (n = 10 per species) and then maintained for 59 d in non contaminated seawater (n = 7 per species).

Uptake parameters. CF_{ss}, concentration factors at steady state; k_u: uptake rate constant (d^{-1})

Depuration parameters. A_{0s} and A_{0l}: activity (%) lost according to the short- and the long-lived exponential component, respectively; $T_{b½}$: biological half-life (d).

ASE: asymptotic standard error; R^2: determination coefficient of the uptake or loss kinetics

	A- Uptake period			B- Loss period				
Isotope	**CF_{ss} ± ASE**	**k_u ± ASE**	**R^2**	**A_{0s} ± ASE**	**$T_{b½s}$ ± ASE**	**A_{0l} ± ASE**	**$T_{b½l}$ ± ASE**	**R^2**
				I. isognomon				
^{51}Cr	229.1 ± 5.7 [b]	10.4 ± 0.9 [d]	0.81			95.7 ± 1.4 [d]	58.2 ± 3.3 [d]	0.76
^{57}Co	976.6 ± 19.3 [d]	33.9 ± 2.5 [d]	0.87	23.8 ± 3.7 [d]	0.8 ± 0.3 [a]	77.1 ± 2.1 [d]	77.8 ± 8.6 [d]	0.74
^{65}Zn		103.2 ± 2.4 [d]	0.85	5.4 ± 2.7 [d]	2.6*	93.8 ± 2.9 [d]	1942*	0.05
^{109}Cd		47.2 ± 1.1 [d]	0.87	12.0 ± 4.8 [d]	0.3*	88.1 ± 2.1 [d]	3933*	0.06
^{110m}Ag		121.2 ± 3.0 [d]	0.85	19.4 ± 3.3 [d]	1.5 ± 0.6 [a]	81.1 ± 2.3 [d]	1559*	0.30
				M. regula				
^{51}Cr	206.6 ± 6.6 [d]	8.2 ± 0.9 [d]	0.73			96.6 ± 1.0 [d]	61.3 ± 2.4 [d]	0.88
^{57}Co	1136.4 ± 24.6 [d]	26.6 ± 2.1 [d]	0.85	25.4 ± 3.2 [d]	0.9 ± 0.3 [a]	74.8 ± 2.2 [d]	74.0 ± 7.9 [d]	0.83
^{65}Zn		98.0 ± 3.2 [d]	0.76	9.3 ± 2.9 [d]	7.3*	88.0 ± 2.7 [a]	842*	0.12
^{109}Cd		35.6 ± 0.75 [d]	0.89	22.1 ± 3.4 [d]	0.3*	77.9 ± 1.6 [d]	330.2 ± 103.4 [b]	0.45
^{110m}Ag		28.2 ± 1.2 [d]	0.66	20.4 ± 5.8 [d]	1.3*	79.4 ± 5.4 [d]	91.1 ± 25.7 [c]	0.58

Probability of the model adjustment: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, [d] $p < 0.0001$; * $p > 0.05$

Table 2. Concentration factors (mean CF ± SD; n = 3 per species) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in the oysters *Isognomon isognomon* and *Malleus regula* after a 28-d exposure via seawater.

Compartments	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
I. isognomon					
in toto	**159 ± 59**	**600 ± 199**	**2,615 ± 871**	**1,178 ± 416**	**3,244 ± 1158**
Shells	233 ± 69	654 ± 67	570 ± 34	493 ± 37	1,319 ± 28
Whole soft parts	46 ± 28	136 ± 23	12,441 ± 3734	4,617 ± 72	5,502 ± 4657
Remaining soft parts	26 ± 4	136 ± 27	14,695 ± 249	4,703 ± 114	14,924 ± 8289
Visceral mass	80 ± 20	155 ± 5	14,494 ± 6637	5,060 ± 699	2,880 ± 949
M. regula					
in toto	**142 ± 54**	**579 ± 147**	**2,754 ± 1108**	**1,074 ± 265**	**783 ± 433**
Shells	211 ± 33	647 ± 92	821 ± 78	659 ± 86	576 ± 52
Whole soft parts	48 ± 29	204 ± 28	33,251 ± 8835	4,293 ± 1205	824 ± 377
Remaining soft parts	38 ± 19	178 ± 2	26,531 ± 3281	4,534 ± 1410	719 ± 422
Visceral mass	93 ± 81	3,179 ± 151	64,005 ± 34,424	3,206 ± 2798	1,265 ± 571

III.2. FEEDING EXPERIMENT

Whole-body loss kinetics were best fitted using a double-exponential model (Table 3 and Fig. 2). In the case of ^{51}Cr, biokinetics could not be determined accurately due to the low activity detectable in clams. A significant fraction (23 to 66 %) of the total metal load ingested with food was associated with the short-lived component of the loss kinetics. This component was characterized by a very rapid loss ($T_{b½s}$ < 1 d). The long-lived component represented 34 to 77 % of ingested metal loads. These assimilated fractions were lost with $T_{b½l}$ ranging from 20 to 394 d (this latter value -^{109}Cd- being not significantly different from ∞). Both species similarly absorbed ^{57}Co and ^{109}Cd from food, whereas *I. isognomon* more efficiently assimilated ^{65}Zn and ^{110m}Ag ($p_{Z\text{-test}} \leq 0.001$ and ≤ 0.02; AE = 77 and 56 %, respectively) than *M. regula* (AE = 57 and 34 %, respectively).

After 72 d of depuration, the distribution of the radiotracers among body compartments was calculated (Table 4). The highest proportion of ^{51}Cr and ^{57}Co was associated with shells in both oysters whereas ^{65}Zn and ^{109}Cd were predominantly associated with the soft parts. In soft parts, the major part of the metals was generally found in the visceral mass, followed by the mantle, muscle, and then gills and palps. In the case of Ag, the distribution between soft parts and shells showed that the major part of the activity was associated with the shells for both *I. isognomon* (60 %) and *M. regula* (89 %). The distribution among soft parts showed that 83 % of Ag load was present in visceral mass in *I. isognomon* whereas in *M. regula* each organ contributed equally to the global metal content.

Figure 2. Loss kinetics of ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in the oysters *Isognomon isognomon* and *Malleus regula* after a 2-hr feeding on radiolabelled *Isochrysis galbana* (Remaining activity -%-; mean ± SD; n = 3 *I. isognomon* and n = 9 *M. regula*).

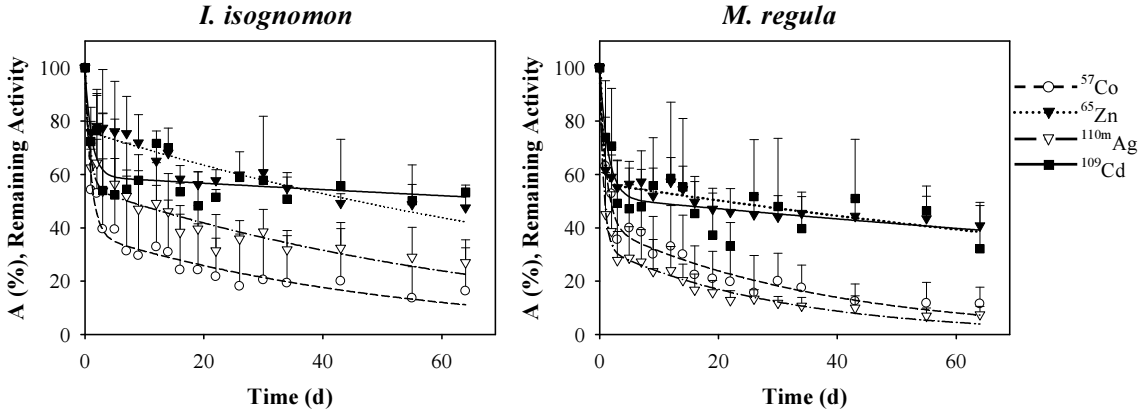

Table 3. Estimated loss kinetic parameters of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in the oysters after a 2-hr feeding on radiolabelled *Isochrysis galbana*, followed by 72 d in non contaminated conditions (n = 3 *Isognomon isognomon* and n = 9 *Malleus regula*).

A_{0s} and A_{0l}: activity (%) lost according to the short-lived (s) and the long-lived (l) exponential component, respectively (A_{0l} is the assimilation efficiency, AE); $T_{b½}$: biological half-life (d); ASE: asymptotic standard error; R^2: determination coefficient of the loss kinetics; n.d.: not determined.

Isotope	A_{0s} ± ASE	$T_{b½s}$ ± ASE	AE ± ASE	$T_{b½l}$ ± ASE	R^2
I. isognomon					
^{51}Cr	n.d.				
^{57}Co	60.3 ± 16.3 ^{c}	0.7*	38.5 ± 11.6 ^{b}	35.0*	0.48
^{65}Zn	22.8 ± 6.1 ^{c}	0.03*	77.2 ± 2.5 ^{d}	72.6 ± 12.6 ^{d}	0.67
^{109}Cd	41.3 ± 7.3 ^{d}	0.8 ± 0.31 ^{a}	58.3 ± 3.6 ^{d}	394 *	0.54
^{110m}Ag	43.2 ± 7.1 ^{d}	0.5*	56.4 ± 4.3 ^{d}	47.1 ± 11.9 ^{c}	0.75
M. regula					
^{51}Cr	n.d.				
^{57}Co	57.9 ± 5.7 ^{d}	0.7 ± 0.2 ^{d}	42.1 ± 4.0 ^{d}	23.8 ± 5.1 ^{d}	0.75
^{65}Zn	42.6 ± 3.3 ^{d}	0.3 ± 0.11 ^{b}	57.4 ± 1.6 ^{d}	100 ± 19 ^{d}	0.70
^{109}Cd	49.2 ± 6.9 ^{d}	1.0 ± 0.3 ^{c}	51.1 ± 3.8 ^{d}	166 *	0.41
^{110m}Ag	66.2 ± 4.0 ^{d}	0.5 ± 0.1 ^{d}	33.6 ± 2.8 ^{d}	19.6 ± 3.6 ^{d}	0.86

Probability of the model adjustment: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$; * $p > 0.05$

Table 4. Radiotracer distribution (mean % ± SD, n = 3 per species) among the different body compartments of the oysters *Isognomon isognomon* and *Malleus regula*, fed 2 hrs on radiolabelled *Isochrysis galbana* and then allowed to depurate for 72 d.

Body compartments	Weight (%)	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
I. isognomon						
Shell	**94 ± 1**	**55 ± 2**	**64 ± 39**	**40 ± 45**	**39 ± 31**	**60 ± 21**
Whole soft parts	**6 ± 1**	**45 ± 2**	**36 ± 39**	**60 ± 45**	**61 ± 31**	**40 ± 21**
Mantle	29 ± 7.5	18 ± 1	20 ± 7	23 ± 1	34 ± 13	4 ± 5
Muscle	29 ± 2	16 ± 1	19 ± 4	13 ± 6	15 ± 3	6 ± 2
Palps	2 ± 1	18 ± 4	17 ± 7	1 ± 1	5 ± 4	4 ± 5
Gills	9 ± 1	19 ± 6	17 ± 2	7 ± 4	5± 3	3 ± 4
Visceral mass	31 ± 5	28 ± 8	28 ± 15	56 ± 10	40 ± 10	83 ± 16
M. regula						
Shell	**96 ± 1**	**48 ± 3**	**87 ± 4**	**31 ± 13**	**32 ± 9**	**89 ± 7**
Whole soft parts	**4 ± 1**	**52 ± 3**	**13 ± 4**	**69 ± 13**	**68 ± 9**	**11 ± 7**
Mantle	21 ± 6	20 ± 1	16 ± 4	13 ± 2	14 ± 2	19 ± 5
Muscle	40 ± 6	19± 1	15 ± 4	11 ± 1	14 ± 5	21 ± 6
Palps	6 ± 1	22 ± 1	15 ± 4	5 ± 3	7 ± 4	19 ± 5
Gills	12 ± 5	13 ± 1	15 ± 5	10 ± 3	10 ± 6	15 ± 6
Visceral mass	21 ± 2	26 ± 3	39 ± 17	61 ± 3	55 ± 12	26 ± 8

III.3. SEDIMENT EXPERIMENT

When oysters were exposed to radiolabelled sediments, whole-body metal uptake kinetics were always best fitted using a saturation model (Table 5 and Fig. 3). Steady-state was reached within 1 to 3 weeks for all studied metals. Kinetic parameters estimated for all metals were not significantly different between *I. isognomon* and *M. regula (*$p > 0.05$). The transfer factor estimated at steady state (TF_{ss}) indicated that bioavailability of sediment-bound metals ranked as: $^{109}Cd \approx {}^{57}Co > {}^{65}Zn > {}^{110m}Ag > {}^{51}Cr$. TF_{ss} values were 4 to 6 orders of magnitude lower than CF_{ss} calculated at the end of the seawater experiment (Table 1 and 5). Comparison between uptake rate constants from seawater and sediment experiments showed that k_u from

sediment was generally 2 to 4 orders of magnitude lower than those measured in seawater experiments.

At the end of the uptake period (35 d), TF were calculated in the different body compartments (Table 6). In both oyster species, the visceral mass showed the highest TF values with maximum values of 26.6 ± 9.6 in *I. isognomon* and 30.6 ± 6.7 in *M. regula* for ^{51}Cr,. The shells contained the highest proportion of metals (63 - 99 %) mainly due to their elevated weight contribution (97 %) to whole-body weight. Among soft parts, the remaining soft parts contained the highest proportion of metals (56 to 95 %).

Figure 3. Uptake and loss kinetics of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in the oysters *Isognomon isognomon* and *Malleus regula* exposed for 35 d to 5 radiotracers via radiolabelled sediments (Transfer Factors -TF-; mean ± SD; n = 10 per species), and then maintained in non contaminated seawater for 49 d (Remaining activity -%-; mean ± SD; n = 7 per species).

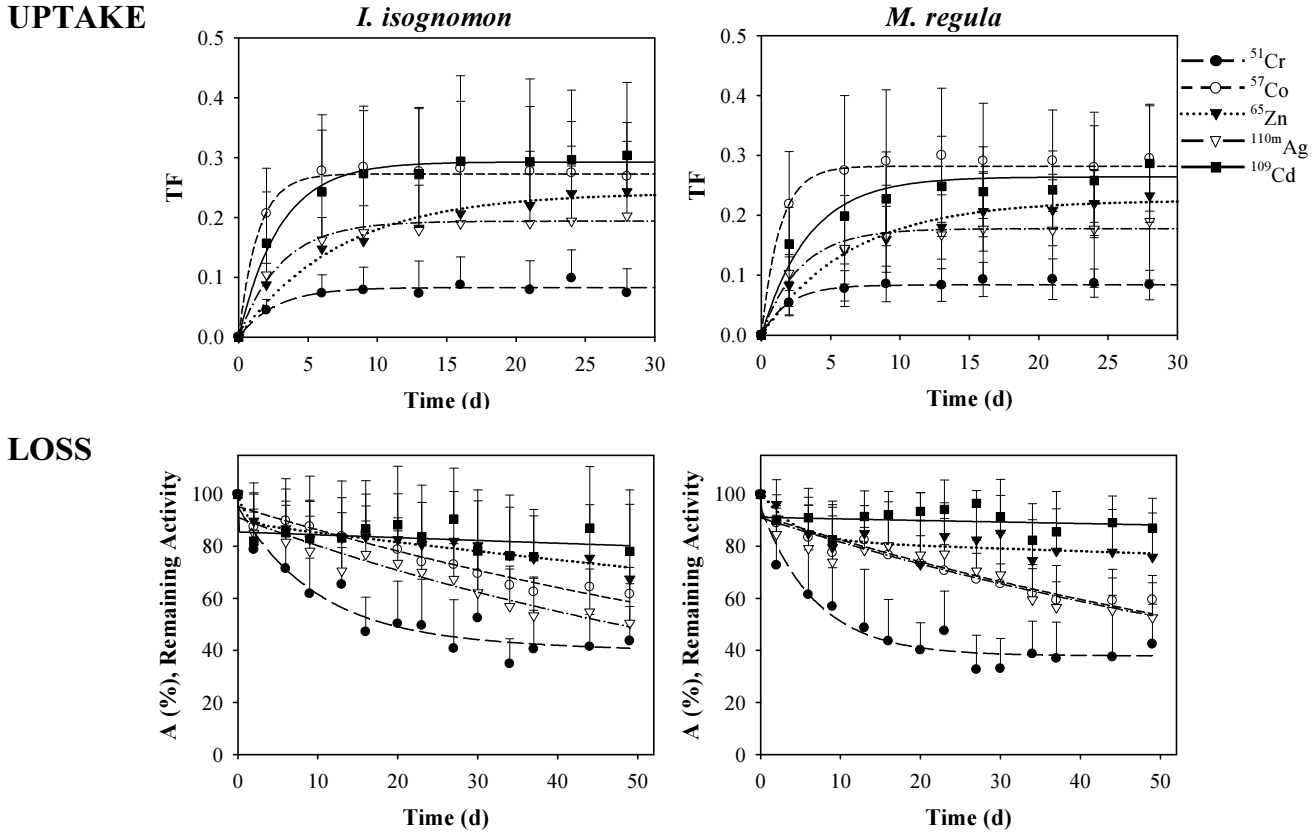

Table 5. Estimated uptake kinetic parameters (A) and loss kinetic parameters (B) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in whole oysters, *Isognomon isognomon* and *Malleus regula* exposed for 35 d via the sediments (n = 10 per species) and then maintained for 49 d in non contaminated seawater (n = 7 per species).

Uptake parameters. TF_{ss}: transfer factors at steady state; k_u: uptake rate constant (d^{-1}).

Depuration parameters. A_{0s} and A_{0l}: activity (%) lost according to the short- and the long-lived exponential component, respectively; $T_{b½}$: biological half-life (d).

ASE: asymptotic standard error; R^2: determination coefficient of the kinetics

	A- Uptake period			B- Loss period				
Isotope	**TF_{ss} ± ASE**	**k_u ± ASE**	**R^2**	**A_{0s} ± ASE**	**$T_{b½s}$ ± ASE**	**A_{0l} ± ASE**	**$T_{b½l}$ ± ASE**	**R^2**
				I. isognomon				
^{51}Cr	0.084 ± 0.003 ^{d}	0.043 ± 0.012 ^{d}	0.47	42.2 ± 12.2 ^{c}	4.3 ± 2.1 ^{a}	56.1 ± 12.3 ^{d}	87.7*	0.66
^{57}Co	0.282 ± 0.010 ^{d}	0.21 ± 0.07 ^{d}	0.43			96.4 ± 1.3 ^{d}	67.2 ± 4.2 ^{d}	0.70
^{65}Zn	0.225 ± 0.010 ^{d}	0.035 ± 0.007 ^{d}	0.62	11.0 ± 5.9	0.7*	89.0 ± 4.2 ^{d}	158.8 ± 62.9 ^{a}	0.22
^{109}Cd	0.264 ± 0.011 ^{d}	0.077 ± 0.023 ^{d}	0.48	14.4 ± 6.6 ^{a}	0.1 *	85.6 ± 4.4 ^{d}	533 *	0.09
^{110m}Ag	0.178 ± 0.010 ^{d}	0.064 ± 0.027 ^{d}	0.29			93.3 ± 2.4 ^{d}	51.3 ± 5.0 ^{d}	0.53
				M. regula				
^{51}Cr	0.083 ± 0.005 ^{d}	0.031 ± 0.012 ^{d}	0.29	59.5 ± 16.2 ^{c}	4.6*	37.7 ± 17.8 ^{a}	2114	0.66
^{57}Co	0.273 ± 0.010 ^{d}	0.20 ± 0.060 ^{c}	0.43			93.5 ± 1.7 ^{d}	60.6 ± 4.6 ^{d}	0.60
^{65}Zn	0.242 ± 0.016 ^{d}	0.033 ± 0.011 ^{c}	0.52	18.5 ± 5.9 ^{b}	2.8 *	82.2 ± 4.9 ^{d}	541*	0.23
^{109}Cd	0.293 ± 0.014 ^{d}	0.099 ± 0.034 ^{d}	0.38	8.8 ± 3.5 ^{a}	1.0*	91.2 ± 2.1 ^{d}	1048*	0.1
^{110m}Ag	0.194 ± 0.011 ^{d}	0.066 ± 0.003 ^{d}	0.30			96.7 ± 2.1 ^{d}	54.4 ± 4.3 ^{d}	0.60

Probability of the model adjustment: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$; * $p > 0.05$

Table 6. Transfer factors (mean TF ± SD; n = 3 per species) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in the oysters *Isognomon isognomon* and *Malleus regula* after 35 d of exposure via sediment.

Compartments	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
I. isognomon					
in toto	**0.072 ±0.032**	**0.260 ±0.099**	**0.227 ±0.071**	**0.303 ±0.124**	**0.184 ±0.115**
Shells	0.544 ±0.144	0.136 ±0.044	0.108 ±0.02	0.126 ±0.043	0.105 ±0.039
Visceral mass	26.6 ±9.6	0.303 ±0.027	3.41 ±1.79	2.65 ±2.45	0.104 ±0.06
Remaining soft parts	1.40 ±0.41	0.047 ±0.006	1.17 ±0.41	1.119 ±1.026	0.022 ±0.000
M. regula					
in toto	**0.082 ±0.043**	**0.248 ±0.099**	**0.244 ±0.085**	**0.304 ±0.152**	**0.200 ±0.123**
Shells	0.340 ±0.066	0.087 ±0.046	0.065 ±0.033	0.077 ±0.038	0.076 ±0.032
Visceral mass	30.6 ±6.4	0.507 ±0.209	2.31 ±1.76	4.012 ±0.992	0.157 ±0.051
Remaining soft parts	1.20 ±0.16	0.045 ±0.004	1.53 ±0.55	0.771 ±0.714	0.034 ±0.018

When oysters were replaced in non-contaminated conditions for 49 d, loss kinetics of ^{51}Cr, ^{65}Zn and ^{109}Cd were best fitted using a double exponential equation whereas loss kinetics of ^{57}Co and ^{110m}Ag were most accurately described by a single exponential equation (Table 5 and Fig. 3). The major part of the total radiotracer activity was generally associated with the long-lived component and was rather strongly retained by oyster tissues ($T_{b½l}$ > 51 d). In particular, ^{51}Cr and ^{109}Cd in both oysters and ^{65}Zn in *M. regula* were very strongly retained with $T_{b½l}$ virtually equal to infinite. In contrast with seawater and food exposures, both oyster species displayed the same behaviour when exposed to Ag via sediment (uptake and loss kinetic parameters not significantly different between the two oyster species). Body distribution of radiotracers at the end of loss period showed that ^{51}Cr, ^{57}Co and ^{110m}Ag were mainly associated with shells (63 - 99 %) whereas ^{65}Zn in both oysters and ^{109}Cd in *I. isognomon* were equally distributed between shells and soft parts.

IV. DISCUSSION

The added radiotracer activities used in the present study corresponded to very low stable metal concentrations (order of pg to ng l^{-1}) hence non significantly departing from realistic *in situ* concentrations. In addition, using γ-emitting radiotracers allowed for radioanalysing organisms without sacrificing them. This provides a series of advantages, such as limiting the number of organisms required and allowing for monitoring metal incorporation and depuration in each individually during the whole experiment duration.

The present study indicated that *I. isognomon* and *M. regula* readily accumulated the five metals tested. In addition, no interspecific difference was found in bioaccumulation and retention behaviour of Co, Cd and Zn except for Ag, which kinetic behaviour strongly differed from one species to the other whenever exposed via seawater or via the food.

IV.1. SEAWATER EXPOSURE

The two oyster species accumulated dissolved metals in a very similar way, except for Ag and to a lesser extend Cd which displayed marked differences. In decreasing order of bioavailability, metals ranked as Ag > Zn > Cd > Co > Cr for *I. isognomon*, and Zn > Cd > Ag > Co > Cr for *M. regula*. The uptake kinetics indicated that Ag, Cd, and Zn were concentrated faster and more efficiently than Cr and Co in both oysters probably due to the high affinity of the three first metals for sulfhydryl groups, which facilitates their association with cellular proteins and transport across cell membranes (e.g., Bryan 1984; Wang et al. 1996). Strong retention of Ag, Cd, and Zn observed during the depuration experiment also indicates that both oyster species evidently possess efficient detoxification processes allowing storage (without toxic effects) of large amounts of these three metals. Such processes could involve binding with metalloproteins, inclusion in lipofuscine matrix or in amorphous granules (Mason & Jenkins 1995; Marigómez et al. 2002).

Once accumulated in oyster tissues, Zn was retained for a very long time ($T_{b½} \simeq \infty$) by the oysters, suggesting that high concentrations of Zn should be found in old oysters. Interestingly, this hypothesis is in perfect agreement with the observations from several field studies that reported very elevated concentrations of Zn in the *Isognomon* genus, reaching 4,010 µg g^{-1} and 12,163 µg g^{-1} dry wt in *I. alatus* from the Dominican Republic (Sbriz et al. 1998) and from Guadeloupe (RNO-Antilles unpublished work), respectively and more than

10,000 µg g^{-1} dry wt in *I. isognomon* from the New Caledonian lagoon (Hédouin et al. submitted-a). Although Zn is an essential element to organisms, e.g. acting as co-factor in numerous metalloenzymes (Vallee & Falchuk 1993), the elevated Zn concentration found in oyster tissues could be due to the immobilization of Zn in phosphate granules which are characterized by very low excretion rate as previously observed in other oyster species (George et al. 1978).

In the case of Cd, elevated concentration factors observed in both oyster tissues could be considered as surprising, due to the known toxicity of this metal (Eisler 1985). It is however well known that Cd(II) can interfere with the metabolism of Ca(II), via Ca^{2+} channels and that Cd(II) displays a high affinity for sulfhydryl groups (Roesijadi & Unger 1993; Williams & Frausto da Silva 1996; Rainbow 1997a). Parameters of Cd loss kinetics indicate that the major part of incorporated Cd (> 82 %) was efficiently retained within the soft tissues of the oysters ($T_{b½}$ > 330 d). The high Cd retention might be related to its binding with metalloproteins (e.g. metallothioneins, MT) and/or its storage in intracellular or intratissular granules (Bebianno & Langston 1992; Mason & Jenkins 1995), as inferred from Zn. Both processes would result in the sequestration of high amounts of Cd in oyster tissues, with limited or no toxic effects.

Ag was the only element which displayed drastic contrasting bioaccumulation behaviour in the two oyster species. On a whole body basis, *I. isognomon* accumulated 4 times more Ag than *M. regula*. This difference in concentration capacity was even more pronounced in the remaining soft parts with CF two orders of magnitude higher in *I. isognomon* than in *M. regula.* Since Ag is a non-essential element and one of the most toxic metals to invertebrates (e.g. Ratte 1999), the particularly high concentration capacity for Ag observed in *I. isognomon* soft tissues probably resulted from sequestration/detoxification processes. Indeed, some mechanisms protecting against Ag are well documented in bivalves. Among these mechanisms, the best known is certainly binding to cystein-rich metalloproteins (e.g. metallothioneins, MT), for which Ag is known to have a very high affinity (Kägi & Kojima 1987; Vasak 1991). Examination of the parameters of Ag loss kinetics after seawater exposure indicated that it was very slowly released from oyster soft tissues: *M. regula* would retain Ag for months ($T_{b½}$ = 91 d) and *I. isognomon* for years ($T_{b½} \simeq \infty$). A MT-based detoxification system could explain quite well the retention capacity observed in *M. regula*. However, given the biological turnover rate of MTs (half-life in *M. edulis* is 25 d for MT-Cd, Bebianno & Langston 1993; and 10 and 60 d for MT-Cu and MT-Zn, respectively, Langston et al. 1998) such a detoxification process would hardly explain the extremely long retention of

Ag in *I. isognomon*. Another detoxification process involving the sequestration of Ag as stable Ag_2S would more consistently explain this unusually high retention capacity. Indeed, it has been documented that bivalves, and particularly oysters, can store large amounts of Ag as Ag_2S in their soft tissues, this non-toxic, insoluble and very stable amorphous material being precipitated and sequestered in the basement membranes and underlying connective tissue for a very long time (e.g. Berthet et al. 1992).

Difference in affinity for Ag was also observed in the shells of both oysters with CF in *I. isognomon* twice those measured in *M. regula* (1,319 vs. 576). It has been previously demonstrated that metals were accumulated differently in the shells of the oysters *Crassostrea gigas* and *C. virginica* depending on the shell quality (Okazaki & Panietz 1981). However, *I. isognomon* and *M. regula* have different shell structures and the former shell-quality hypothesis is not supported by our measured CF values. Indeed, the multilayered structure of *M. regula* shell would favor higher Ag adsorption compared to *I. isognomon*, which is not the case. Alternatively, the difference in shell CF could be at least partly due to different calcification patterns in both species. For instance, the nacreous formation is much more abundant in *I. isognomon* than in *M. regula* (Yonge 1968)

In contrast to elements with S-affinity, metals displaying O-affinity (Co and Cr) (Nieboer & Richardson 1980) were concentrated much more efficiently in shells than in soft tissues of both oysters. In terms of relative distribution, 96 - 98 % of the total activity of Co and Cr were associated with the shells. This predominant distribution is in accordance with the observations reported in several other marine organisms, in which adsorption of Co and Cr onto the shell is the major process of accumulation (e.g. Fisher et al. 1996; Hutchins et al. 1998). Loss kinetics of Cr and Co were characterized by turnover times ($T_{b½}$ = 58 to 78 d) faster than those of the other metals examined in oysters, which is in agreement with the fact that desorption processes are generally faster than elimination of biologically-incorporated elements.

IV.2. FOOD EXPOSURE

In oysters exposed via the food chain, whole-body loss kinetics were best described by a double-component exponential model for all metals tested (in the case of Cr, the ^{51}Cr activity measurements prevented establishing reliable loss kinetics). An important fraction (23 to 66 %) of ingested metals was rapidly lost, with a $T_{b½}$ < 1 d. This short-lived component corresponded to the elimination of the fraction of metals ingested with food that remained

associated with, and was eliminated with the faeces and/or pseudofaeces. The second long-lived exponential component of the loss kinetics showed that 34 to 77 % of the metals ingested were actually absorbed by the oysters and rather strongly retained within their tissues ($T_{b½}$ ranging from 20 d to ∞). The AEs of Ag, Cd, Co and Zn measured in both oysters were comparable to their respective AEs assessed in several other bivalves species (e.g. Reinfelder et al. 1997). Among them, Zn was generally assimilated with the highest efficiency (AE = 77 % in *I. isognomon* and 57 % in *M. regula*). High Zn assimilation has been also measured in other species such as the clam *Mercenaria mercenaria* (AE = 86 %) or the oyster *Crassotrea virginica* (AE = 73 %) (Reinfelder et al. 1997). Indeed, it has been generally observed that essential elements are assimilated with a higher efficiency than non essential elements (e.g., Wang & Fisher 1999a) although exceptions exist (e.g., methylmercury).

Both oysters displayed similar loss kinetics for Cd, Co and Zn ingested with food, with turnover time ranging from 166 to 394 d (not significantly different from the infinite), 24 to 35 d and 73 to 100 d, respectively. In contrast, and similarly to what was observed for seawater experiments, kinetic behaviour of dietary Ag was specific for each oyster species: Ag was more strongly retained in *I. isognomon*, with $T_{b½}$ twice those measured in *M. regula.* This further supports the hypothesis according to which different detoxification mechanism(s) occurred in each of the two oysters. However, unlike Ag retention after seawater exposure, dietary Ag was not found to be sequestered in the tissues of *I. isognomon*, hence suggesting the dominance of dissolved vs. food Ag contamination pathway in the oyster, as previously suggested for other marine invertebrates (Berthet et al. 1992; Warnau et al. 1996b; Shi et al. 2003).

IV.3. SEDIMENT/PARTICULATE MATERIAL EXPOSURE

In New Caledonia, contamination mainly arises from land-based mining activities delivering metal-rich particles to the lagoon and jointly enhancing water turbidity (Laganier 1991). Consequently, metal concentrations in sediment, which are generally much higher than those in seawater may represent an important source of metals for marine organisms (Luoma 1990).

Contrasting results are reported in the literature regarding the impact of sediment pathway as a source of metal uptake in marine organisms. For example, it has been reported that sediment was the main contamination pathway for Cd in the deposit-feeding polychaete *Capitella capitata* (Selck et al. 1998) whereas this pathway was far less important than seawater for Cd accumulation in the infaunal cockle *Anadra trapezium* (Scanes 1993). In the present study,

whole-body uptake kinetics of the five metals examined rapidly reached a steady-state equilibrium and similar bioaccumulation patterns were found for both oyster species (including Ag). Estimated TF_{ss} were 4 to 6 orders of magnitude lower than CF_{ss} calculated in the seawater experiment, indicating that sediment-bound elements were far less bioavailable for oysters than metals dissolved in seawater. However, our study demonstrated that Ag, Cd, Co and Zn bound to sediment particles were assimilated in both oyster species with a higher efficiency (A_{0l} from 82 to 97 %) than phytoplankton-bound metals (AE from 33 to 77 %). This result is surprising as metal assimilation efficiencies from ingested sediment generally tend to be lower than those from ingested phytoplankton (Gagnon & Fisher 1997; Chong & Wang 2000b). Such an efficient assimilation of sediment-bound metals could be related to the geochemical properties of sediment, which have been shown to be critical in affecting metal bioavailability (Luoma 1989; Griscom et al. 2000), and/or to efficient desorption of sediment-bound metals within the gut of the studied oysters as it is acknowledged as a major step controlling metal bioavailability to organisms (Mayer et al. 1996).

IV.4. CONCLUSIONS

Identification of bioindicator species of metal contamination is of major importance to environmental management of the New Caledonia lagoon subject to increasing anthropogenic pressure. Our results showed that the oysters *Isognomon isognomon* and *Malleus regula* efficiently accumulated Ag, Co, Cd, Cr and Zn suggesting that valuable information on metal contamination can be gained from the periodical monitoring of their body concentrations. In addition, both oyster species efficiently retained metals ($T_{b½}$ generally ≥ 1 month), indicating that they are able to integrate and preserve information over a long period of time.

I. isognomon and *M. regula* could be used as quasi-interchangeable biomonitors of mining-originating metal contamination due to their very similar bioaccumulation capacities for 4 out of the 5 metals tested. The only exception was Ag for which the two oysters displayed contrasting bioaccumulation patterns. Although Ag is probably not a major contaminating element in the New Caledonia coastal waters, it is known as a reliable indicator of domestic sewage inputs (Sanudo-Willhelmy & Flegal 1992). Therefore, monitoring Ag levels in oysters is of additional value as it would represent a good indicator of long-term urban and anthropogenic impacts. In this respect, *I. isognomon* could provide information on Ag contamination levels integrated over the whole lifespan of the species, whereas *M. regula* would be able to provide information on a shorter time-scale.

It has to be stressed that the present experimental study used low background metal concentrations to determine biokinetic characteristics of the metal investigated. Even though data are still very scarce, reported metal concentrations in the New Caledonia waters and sediments vary over a wide range (Fernandez et al. 2002a; Fernandez et al. 2006; Hédouin et al. submitted-a) and concentrations should increase due to ongoing mining and processing developments. Therefore, further studies are required to investigate the potential effects of highly contrasting exposure concentrations on metal bioaccumulation in oysters in order to reinforce their validity as sentinels of ambient metal contamination.

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CHAPITRE 3

Bio-cinétiques des métaux dans le clam tropical *Gafrarium tumidum* exposé aux métaux via l'eau de mer, les sédiments et la nourriture

Le clam *Gafrarium tumidum* a été étudié afin d'évaluer son utilité en tant qu'espèce bioindicatrice de la contamination métallique due aux activités minières dans le lagon de Nouvelle-Calédonie. Les cinétiques d'accumulation et d'élimination du Cr, Co, Zn, Cd et de l'Ag par l'intermédiaire de l'eau de mer, du sédiment et de la nourriture ont été déterminées dans le clam à l'aide des techniques extrêmement sensibles des radiotraçage (^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd et ^{110m}Ag). Lorsque les clams sont exposés aux métaux dissouts, le Co, le Zn et l'Ag sont rapidement incorporés dans leurs tissus (facteurs de concentration - FC - de 181 à 4.982 après 28 jours d'exposition) et tous les métaux sont retenus de manière efficace (temps de demie vie biologique - $T_{b½}$ - toujours > 2 mois). Après une exposition de 35 jours aux métaux par l'intermédiaire des sédiments, le facteur de transfert estimé - FT - dans les tissus du clam est 1 à 4 ordres de grandeur plus faibles que les FC estimés, suggérant que la biodisponibilité des métaux liés aux sédiments est plus faibles que celle des métaux dissouts. Les métaux liés aux sédiments sont absorbés avec une meilleure efficacité (58 à 91 %) que les métaux ingérés avec la nourriture (36 à 79 %). De plus, une fois absorbés, les métaux incorporés via l'eau de mer et les sédiments sont retenus plus efficacement que les métaux ingérés avec la nourriture. Ceci suggère que la voie d'accumulation peut influencer les processus de stockage des métaux dans les tissus du clam.

Les importantes capacités de bioaccumulation et de rétention du clam *G. tumidum* indiquent que son utilisation en tant qu'espèce bioindicatrice dans un programme de biosurveillance serait intéressante pour obtenir des informations sur les niveaux ambiants de la contamination minière dans l'environnement marin.

Biokinetics of metals in the tropical marine clam *Gafrarium tumidum* exposed via seawater, sediments and food pathways

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To be submitted

ABSTRACT. The edible clam *Gafrarium tumidum* was investigated to assess its usefulness as bioindicator species of metal contamination from mining origin in the New Caledonia lagoon. The uptake and loss kinetics of Cr, Co, Zn, Cd and Ag were determined following exposures via seawater, sediments and food using highly sensitive radiotracer techniques (^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag). When the clams were exposed to dissolved metals, Co, Zn and Ag were readily incorporated in their tissues (concentration factors - CF - ranging from 181 to 4,982 after 28 d of exposure) and all metals were strongly retained (biological half-lives -$T_{b½}$- always > 2 months). The estimated transfer factor - TF - in clam tissues after a 35-d sediment exposure was 1 to 4 orders of magnitude lower than the estimated CF, suggesting the bioavailability of sediment-bound metals was lower than that of the dissolved metals. Sediment-bound metals were absorbed with higher efficiency (58 to 91 %) than metals ingested with food (36 to 79 %). Once absorbed, metals taken up from sediment and seawater were retained for longer than metals ingested with food, indicating that the uptake pathway influences the storage processes of metals in clam tissues. The efficient bioaccumulation and retention capacities of the clam *G. tumidum* indicate that its use in a biomonitoring programme as bioindicator species would be interesting to furnish information on ambient contamination levels in the marine environment.

Keywords: Bioaccumulation, Radiotracers, Seafood, Mining Activities

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I. INTRODUCTION

New Caledonia mineral resources (mainly Co, Cr, Fe, Mn and Ni) have been exploited for more than a century (Dalvi et al. 2004) and presently constitute the major economical resource of the Territory. The development of human activities in coastal zones leads to important perturbation of marine ecosystems and could represent a threat to coral reef ecosystems (Labrosse et al. 2000). Even though ecotoxicological data relating to the lagoon of New Caledonia are scarce (e.g. Monniot et al. 1994), the contamination of the South-West lagoon from both mining (e.g. Co, Cr, Mn and Ni) and urban activities (Ag, Cd) is now well established (Labrosse et al. 2000; Breau 2003; Hédouin et al. submitted-a). Moreover, recent developments of a new extraction process of Co and Ni based on acidic extraction (lixiviation) are expected to be applied at the industrial scale in the very near future in the South of the Territory (Goro-Nickel 2001). Although such process gives the opportunity to extract Ni, and to a lesser extend Co, from ores with lower Ni contents than those currently exploited (Mihaylov et al. 2000; Goro-Nickel 2001; Dalvi et al. 2004), this process is an issue of concern. Indeed, the acidic solubilization is obviously not restricted to Ni but also concerns all other ore-contained by-product metals. This will therefore lead to important additional discharges of dissolved by-product metals into the marine environment (Goro-Nickel 2001; Baroudi et al. 2003).

In this context, there is an urgent need to determine reliable tools to assess and monitor the metal contamination in New Caledonia coastal waters. The usefulness of bioindicator species is a valuable and informative approach, which allows determining the bioavailable fraction of metals present in the marine environment (e.g. Phillips 1990). A great attention has been paid to the implementation of biomonitoring programmes in temperate areas using bivalves such as the mussel *Mytilus edulis* in the Mussel Watch (e.g. O'Connor 1998). Conversely, only very few studies have addressed this issue in tropical areas. In addition, available studies recommend the use of the green mussel *Perna viridis* or the clam *Ruditapes philippinarum* (Rainbow & Phillips 1993; Ng & Wang 2004). However, none of these species is found in sufficient abundance in New Caledonia to be used as bioindicator. Aiming at implementing a biomonitoring programme in New Caledonia, a recent work has screened the metal contamination levels in a variety of local marine organisms from different sampling sites of the southwest lagoon (Breau 2003). Among these species, the brown alga *Lobophora*

variegata, the oysters *Isognomon isognomon* and *Malleus regula*, and the clam *Gafrarium tumidum* fulfil several criteria allowing them to be considered as valuable bioindicator candidates (e.g. Phillips 1976a, 1990). Indeed, they are abundant, widely distributed in the lagoon as well as in other tropical ecosystems (Baron & Clavier 1992b) and easy to collect. Recent radiotracer experiments have shown the efficient bioaccumulation capacities of metals in the brown alga *L. variegata* (Metian et al. to be submitted) and in the oysters *I. isognomon* and *M. regula* (Hédouin et al. to be submitted-b), but information relative to metal bioaccumulation in the clam *G. tumidum* is lacking.

As a seafood, the clam *G. tumidum* is consumed by local populations, and might be considered as a source of exposure for Man. Consequently, its use as quantitative bioindicator could also be interesting for gathering health related information.

The aim of the present study was to assess the usefulness of the clam *Gafrarium tumidum* as a bioindicator species of metal contamination in the New Caledonia coastal waters. Therefore the bioaccumulation capacities of the species was determined by exposing clams via different pathways (seawater, sediments and food) to 4 of the main by-product metals found in Ni ores (Cd, Co, Cr, and Zn), and to Ag which is a reliable proxy for domestic inputs in coastal waters (Sanudo-Willhelmy & Flegal 1992).

II. MATERIALS & METHODS

II.1. COLLECTION AND ACCLIMATION

The clams *Gafrarium tumidum* were collected by sea-shore fishing in September 2002 in Ouano Beach, Nouméa, New Caledonia. Body size is known to affect metal concentrations in organisms (e.g. Boyden 1977; Hédouin et al. in press). Therefore, only individuals with shell width > 35 mm were considered for the experiments.

Individuals were transported to IAEA-MEL premises in Monaco, where they were acclimated to laboratory conditions simulating those of the New Caledonian waters (open circuit 3000 l aquarium; natural seawater flux: 300 l h^{-1}; salinity: 36 p.s.u.; temperature T°: 25 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) for 2 months prior to experimentations. During this period, clams were daily fed a mixed algal diet (*Isochrysis galbana, Heterocapsa triquetra, Thalassiosira pseudonana, Emiliania huxleyi*).

II.2. RADIOTRACERS AND COUNTING

Investigated elements (Ag, Cd, Co, Cr and Zn) were introduced into the experimental microcosms as radiotracers of high specific activity purchased from Amersham, UK (^{51}Cr as Na_2CrO_4, $T_{½}$ = 27.7 d and ^{57}Co as $CoCl_2$, $T_{½}$ = 271.8 d), CERCA, France (^{110m}Ag as $AgNO_3$, $T_{½}$ = 249.8 d), and Isotope Product Lab., USA (^{109}Cd as $CdCl_2$, $T_{½}$ = 426.6 d and ^{65}Zn as $ZnCl_2$, $T_{½}$ = 243.9 d). Radioactivity was measured using a high-resolution γ-spectrometer system composed of 3 Germanium -N or P type- detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser (Intergamma, Intertechnique). The radioactivity of the samples was determined by comparison with standards of known activities and of appropriate geometry. Measurements were corrected for counting efficiency, background and radioactive decay. Counting times were adapted to obtain counting rates with propagated errors less than 5%.

II.3. EXPERIMENTAL PROCEDURE

II.3.1. Exposure via seawater

Twenty clams (whole-body wet wt from 11 to 19 g) were placed in a 70 l glass aquarium (salinity: 36 p.s.u.; T°: 25 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) and exposed to the 5 radiotracers dissolved in seawater for 28 d. The activity of each selected radiotracer was: ^{51}Cr (3 kBq l^{-1}), ^{57}Co (1 kBq l^{-1}), ^{65}Zn (0.5 kBq l^{-1}), ^{109}Cd (3 kBq l^{-1}) and ^{110m}Ag (0.5 kBq l^{-1}). In terms of stable metals addition, these activities corresponded to Cr (0.3 ng l^{-1}), Co (55 pg l^{-1}), Zn (6 ng l^{-1}), Cd (0.15 ng l^{-1}) and Ag (21 ng l^{-1}), which are 1 to 3 orders of magnitude lower than concentrations commonly reported in the natural seawater (Bruland 1983).

No change in pH was detectable after radiotracer addition. Seawater and spikes were renewed daily for 15 d and then every second day in order to keep activities in seawater as constant as possible. Activities of the radiotracers in seawater were checked daily, and before and after each spike renewal in order to determine the time-integrated activities (Warnau et al. 1996b). For the entire experimental time course, the time-integrated radiotracer activities in seawater were ^{51}Cr (2.9 ± 0.2 kBq l^{-1}), ^{57}Co (0.8 ± 0.2 kBq l^{-1}), ^{65}Zn (0.5 ± 0.2 kBq l^{-1}), ^{109}Cd (2.7 ± 0.8 kBq l^{-1}) and ^{110m}Ag (0.4 ± 0.2 kBq l^{-1}). During the experiment, clams were collected at different time intervals and were whole-body γ-counted. At the end of the exposure time (28 d), 5 clams were collected and shells, digestive gland and remaining soft parts were separated,

weighed and radioanalysed in order to assess the metal distribution among these body compartments.

The 15 remaining clams were then placed for 59 d in non-contaminated seawater (open circuit, natural seawater flux: 50 l h^{-1}; salinity: 36 p.s.u.; T°: 25 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). At different times, clams were γ-counted in order to follow the whole-body loss kinetics of the radiotracers. At the end of the depuration period (59 d), 5 individuals were collected and dissected to determine the distribution of radiotracer contents among the different body compartments.

II.3.2. Exposure via sediment

Sediments (3 kg) collected in September 2002 from Sainte-Marie Bay, New Caledonia were placed for 7 d in 3 l of seawater and spiked with the 5 radiotracers (^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag) using the same activities as those used in seawater experiment: ^{51}Cr (3 kBq l^{-1}), ^{57}Co (1 kBq l^{-1}), ^{65}Zn (0.5 kBq l^{-1}), ^{109}Cd (3 kBq l^{-1}) and ^{110m}Ag (0.5 kBq l^{-1}). The spike was renewed daily. Thereafter, sediments were placed in a 70 l aquarium to form a continuous layer of 2 cm height and open circuit conditions were restored (natural seawater flux: 50 l h^{-1}; salinity: 36 p.s.u.; T°: 25 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). Weakly bound metals were allowed to leach overnight under flowing seawater. The clams *G. tumidum* (n = 20, whole-body wet wt from 13 to 19 g) were then placed for 35 d in the aquarium (open circuit, natural seawater flux: 50 l h^{-1}; salinity: 36 p.s.u.; T°: 25 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) on the radiolabelled sediments. The individuals were γ-counted at different time intervals. At the end of uptake period (35 d), 5 clams were collected and their digestive gland and remaining soft parts were separated from shells, weighed and counted in order to determine the distribution of radiotracer contents among these different body compartments. After 35 d, remaining individuals (n = 15) were transferred for 49 d to a new aquarium containing clean flowing seawater (open circuit, natural seawater flux: 50 l h^{-1}; salinity: 36 p.s.u.; T°: 25 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). At the end of loss period (49 d), 5 clams were collected and dissected as previously described to determine the body distribution of the radiotracers.

II.3.3. Exposure via food

Cells of the Prymnesiophyceae *Isochrysis galbana* from an axenic stock culture were resuspended into an erlenmeyer flask (10^3 cell ml^{-1}) containing 5 l of sterile-filtered seawater (0.22 µm) enriched with f/2 nutrients without EDTA and Si. The culture was spiked using the

same activities as those used in seawater experiment: ^{51}Cr (3 kBq l^{-1}), ^{57}Co (1 kBq l^{-1}), ^{65}Zn (0.5 kBq l^{-1}), ^{109}Cd (3 kBq l^{-1}) and ^{110m}Ag (0.5 kBq l^{-1}) and the culture was then incubated for 6 d (light/dark cycle: 12 hrs/12 hrs at 25°C). After incubation, the cell density was 1.2 10^6 cell ml^{-1}. The cells were then gently centrifuged at 2500 × g for 20 min in a Sorvall RC28S ultracentrifuge and the radioactivity of the radiolabelled *I. galbana* was γ-counted before and after the cellular centrifugation.

Twelve clams (whole-body wet wt from 18 to 29 g) were placed in a 70 l glass aquarium (close circuit aquarium constantly aerated, salinity: 36 p.s.u.; T°: 25 ± 0.5°C; pH: 8.0 ± 0.1) and fed the radiolabelled *I. galbana* for 2 hrs (10^4 cell ml^{-1}). Control empty shells were placed in each aquarium to check for any direct uptake of radiotracers from seawater due to possible radiotracer leaching from phytoplankton cells during the feeding period. These control shells were radioanalysed at regular intervals of time.

Immediately after the feeding period, all clams were γ-counted and open circuit conditions were restored (natural seawater flux: 50 l h^{-1}; salinity: 36 p.s.u.; T°: 25 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). Then, at different time intervals, all individuals were γ-counted to determine the whole-body loss kinetics of the radiotracers ingested with food. At the end of depuration period (72 d), 3 individuals were collected and dissected to determine the distribution of radiotracer contents among the different body compartments (gills, mantle, adductor muscle, foot, digestive gland, remaining soft parts and shells).

II.4. DATA ANALYSES

Uptake of the five investigated radiotracers was expressed in terms of concentration factor, CF (viz. the ratio between whole body activity -Bq g-1 wet wt- and time-integrated activity of radiotracers in seawater -Bq g-1-). Radiotracer uptake kinetics were described using either a simple linear regression model (eq.1) or a simple exponential kinetic model (eq.2) when observed kinetics tended to reach a steady state:

$CF_t = k_u\ t$ (eq.1)

$CF_t = CF_{ss}\ (1\text{-}e^{-k_e\ t})$ (eq.2)

where CF_t and CF_{ss} ($CF_{ss} = k_u/k_e$) are the concentration factors at time t (d) and at steady state, respectively; k_u and k_e are the uptake and loss rate constants (d^{-1}), respectively (Whicker & Schultz 1982).

Loss of radiotracers was expressed using the percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in organisms at the beginning

of the decontamination period). Loss kinetics were described by either a single-component (eq.3) or a double-component exponential loss model (eq.4):

$A_t = A_0\ e\text{-}k_e\ t$ (eq.3)

$A_t = A_{0s}\ e^{-k_{es}\ t} + A_{0l}\ e^{-k_{el}\ t}$ (eq.4)

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; ke is the depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components. For each exponential component (s and l), a biological half-life can be calculated ($T_{b½s}$ and $T_{b½l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b½} = \ln 2/k_e$. Regarding feeding experiments, the 'long-lived' exponential term describes the proportion of the radiotracer ingested with food that is actually absorbed by the organism and slowly eliminated. The corresponding A_{0l} represents the assimilation efficiency (AE) of the considered element.

Constants of the models and their statistics were estimated by iterative adjustments of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica® 5.2.1 software. Best fitting regression models were selected according to highest determination coefficient and examination of residuals. The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

III.1. EXPOSURE VIA SEAWATER

The whole-body uptake kinetics in the clam *Gafrarium tumidum* were best described by a simple linear regression for ^{51}Cr and ^{109}Cd and by a saturation exponential equation for ^{57}Co, ^{65}Zn and ^{110m}Ag (Table 1, Fig. 1A). Estimated uptake rate constants (k_u) indicated that metals are accumulated in the organisms with different efficiencies varying from 1 to 2 orders of magnitude (Table 1).

After 28 d of exposure, the radiotracer concentration factors ($CF_{28\ d}$) calculated in whole-body clams indicated that the bioavailability of the dissolved metals ranked as follows: ^{110m}Ag > ^{65}Zn > ^{57}Co > ^{109}Cd ≈ ^{51}Cr (Table 2).

Table 1. Estimated uptake kinetic parameters (A) and loss kinetics parameters (B) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in whole-body *Gafrarium tumidum* exposed to radiotracers via seawater for 28 d (uptake period; n = 20 except for ^{110m}Ag for which four clams were considered individually) and then maintained for 59 d in non contaminated seawater (depuration period; n = 15 except for ^{110m}Ag for which n = 13).

Uptake parameters. CF_{ss}: concentration factors at steady state; k_u: uptake rate constant (d^{-1}).

Depuration parameters. A_{0s} and A_{0l} : activity (%) lost according to the short- and the long-lived exponential component, respectively; $T_{b½}$: biological half-life (d).

ASE: asymptotic standard error; R^2: determination coefficient of the kinetics

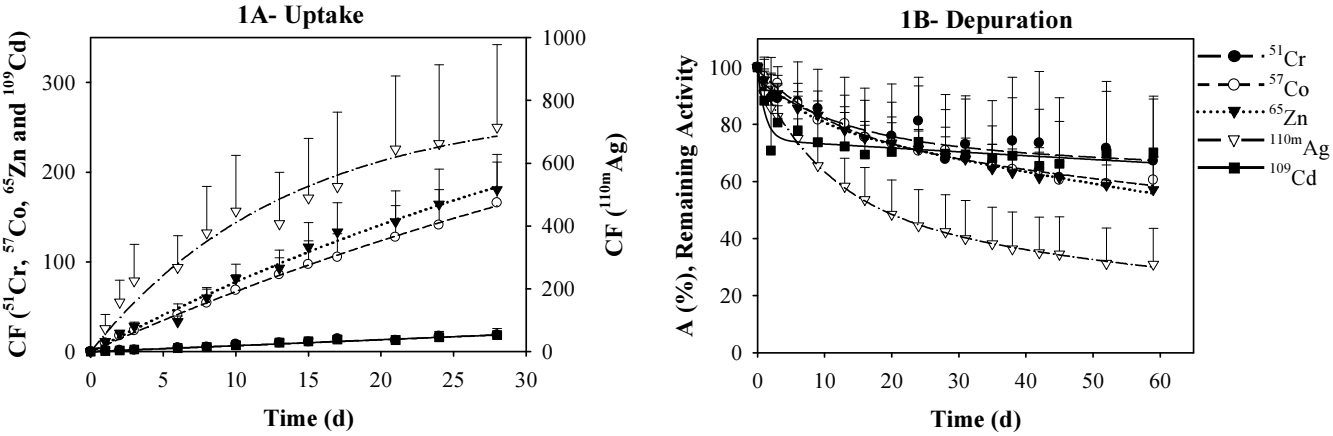
	A- Uptake period			B- Depuration period				
Isotopes	**CF_{ss} ± ASE**	**k_u ± ASE**	**R^2**	**A_{0s} ± ASE**	**$T_{b½s}$ ± ASE**	**A_{0l} ± ASE**	**$T_{b½l}$ ± ASE**	**R^2**
^{51}Cr		0.700 ± 0.015 ^{d}	0.75	22.5 ± 35.6 *	8.9 ± 18.8 *	73.2 ± 38.0 *	474 ± 3,069 *	0.18
^{57}Co	430 ± 152	7.3 ± 0.4 ^{d}	0.83	22.9 ± 17.9 *	7.3 ± 8.0 *	76.4 ± 19.4 ^{c}	152 ± 169 *	0.84
^{65}Zn	429 ± 112	8.6 ± 0.4 ^{d}	0.86	15.5 ± 3.5 ^{d}	4.1 ± 2.2 *	83.1 ± 4.1 ^{d}	102 ± 18.1 ^{d}	0.81
^{109}Cd		0.684 ± 0.015 ^{d}	0.75	25.6 ± 5.3 ^{d}	0.7 ± 0.4 ^{a}	74.9 ± 2.9 ^{d}	336 ± 205 *	0.18
^{110m}Ag	785 ± 177	58.2 ± 6.2 ^{d}	0.61	47.7 ± 15.6 ^{b}	7.0 ± 3.0 ^{a}	49.7 ± 16.6 ^{b}	80 ± 63 *	0.83
n° 9	21,282 ± 7,217	270 ± 11.9 ^{d}	0.99			99.5 ± 1.0 ^{d}	708 ± 256 ^{a}	0.31
n°14	4,261 ± 2,485	70.5 ± 6.7 ^{d}	0.97			98.7 ± 1.1 ^{d}	61 ± 13 ^{b}	0.85
°16	3,711 ± 1,203	98.7 ± 7.8 ^{d}	0.98					
n°18	9,773 ± 5,011	154 ± 12.4 ^{d}	0.98					

Probability of the model adjustment: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$, * $p > 0.05$

Table 2. Concentration factors (CF, mean ± SD; n = 5, except for ^{110m}Ag n = 3) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* exposed for 28 d via seawater.

Body compartments	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
in toto	**18.6 ± 7.0**	**166 ± 45**	**180 ± 39.5**	**18.4 ± 4.6**	**714 ± 263**
Shells	**25.7 ± 11.1**	**177 ± 54**	**80.1 ± 6.3**	**13.1 ± 4.9**	**173** ± 64.5
Whole soft parts	**33 ± 12.6**	**181 ± 73.2**	**629 ± 99.8**	**43.8 ± 12.4**	**4,982** ± 536
Digestive gland	141 ± 87.5	607 ± 437	942 ± 368	83.3 ± 52.8	10,938 ± 3,816
Remaining soft parts	18.1 ± 7.2	120 ± 37.9	587 ± 78.6	38.2 ± 8	3,065 ± 382

Figure 1. Uptake and loss kinetics of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* (1A) exposed for 28 d via seawater (mean concentration factors, CF, ± SD; n = 20 except for ^{110m}Ag, for which clams n° 9, 14, 16 and 18 were considered individually), and then (1B) maintained for 59 d in non contaminated seawater (% remaining activity, A, mean ± SD; n = 15 for all radiotracers except for ^{110m}Ag, n = 13).



Among the soft tissues, all investigated metals showed the highest CFs in the digestive gland (Table 2). ^{110m}Ag was the best bioconcentrated element in whole-body clams (CF = 714), shells (CF = 173) and in clam tissues (the remaining soft part parts reaching CF = 3,065 and the digestive gland CF = 10,938). Interestingly, 4 clams (number 9, 14, 16 and 18) displayed a contrasted behaviour compared to the other organisms with clearly higher bioconcentration capacities of ^{110m}Ag (Table 1, Fig. 2). Thus, these 4 individuals were considered separately and their kinetic models were estimated individually (Table 1, Fig. 2). Due to their very high estimated whole-body concentration factors at steady state (CF_{ss} ranging from 3,711 to 21,282), they can be considered as hyper-accumulator individuals for ^{110m}Ag.

Metal distribution between soft tissues and shells indicated that the major part of ^{51}Cr and ^{57}Co activity was associated with the shells (78 to 83 %) whereas 40 to 60 % of ^{65}Zn, ^{109}Cd and ^{110m}Ag were found in the soft tissues, and especially in the remaining soft parts (Table 3).

Figure 2. Individual uptake kinetics of ^{110m}Ag in 4 hyper-accumulator *Gafrarium tumidum* exposed for 28 d via seawater (concentration factors, CF, n = 1 except the reference kinetics of "normal" clams, n =16).

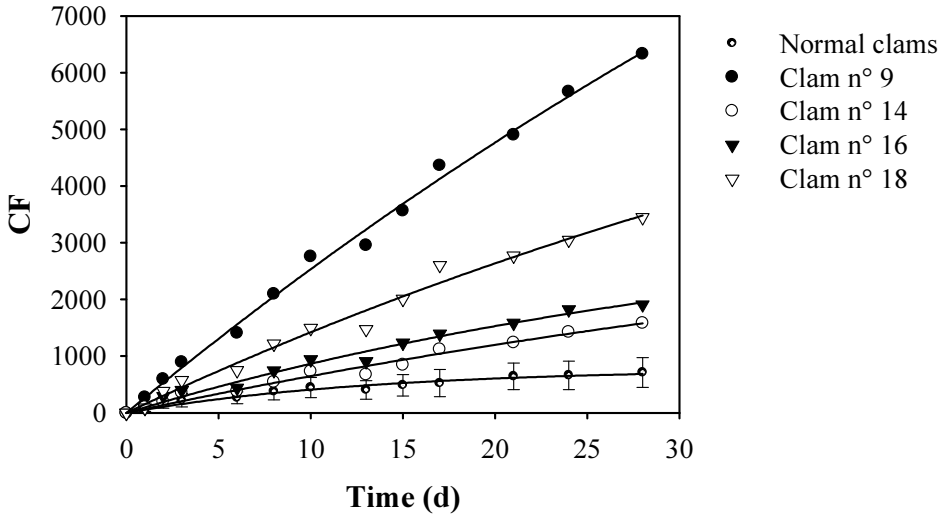


Table 3. Body distribution (mean % ± SD, n = 5 except for ^{110m}Ag n = 3) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* at the end of the 28-d seawater exposure (A) and at the end of the subsequent 59-d depuration period (B).

Body compartments	Weight (%)	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
A- Uptake period (28 d)						
Shells	**83.9 ± 0.9**	**77.6 ± 14.5**	**82.9 ± 9.4**	**40.2 ± 4.2**	**59.6 ± 15.9**	**15.2 ± 3.3**
Whole soft parts	**16.1 ± 0.86**	**22.4 ± 14.5**	**17.1 ± 9.4**	**59.8 ± 4.2**	**40.4 ± 15.9**	**84.8 ± 3.3**
Digestive gland	13.4 ± 4.6	51.1 ± 13.8	39.2 ± 13.2	18.9 ± 4.4	22.6 ± 10.5	29.9 ± 5.5
Remaining soft parts	86.6 ± 4.6	48.9 ± 13.8	60.8 ± 13.2	81.1 ± 4.4	77.4 ± 10.5	70.1 ± 5.5
B- Depuration period (59 d)						
Shells	**89.0 ± 2.3**	**76.0 ± 21.5**	**89.9 ± 0.7**	**33.9 ± 7.3**	**14.0 ± 5.7**	**28.3 ± 5.2**
Whole soft parts	**11.0 ± 2.3**	**24.0 ± 21.5**	**10.1 ± 0.7**	**66.1 ± 7.3**	**86 ± 5.7**	**57.6 ± 5.2**
Digestive gland	13.4 ± 7.8	37.9 ± 7.5	47.9 ± 21.1	23.2 ± 6.5	22.1 ± 11.3	25.9 ± 18.1
Remaining soft parts	86.6 ± 7.8	62.1 ± 7.5	52.1 ± 21.1	76.8 ± 6.5	77.9 ± 11.3	74.1 ± 18.1

At the end of the uptake period, non-contaminated conditions were restored and whole-body loss kinetics of radiotracers were followed for 59 d (Fig. 1B). Loss kinetics were best fitted by a double exponential model for all the radiotracers investigated (Table 1). The short-lived components concerned 16 - 48 % of the total radioactivity and was rapidly eliminated ($T_{b½s}$ < 9 d). The long-lived component represented the major part of incorporated radiotracers (A_{0l} = 50 - 83 %) that was efficiently retained in whole clams ($T_{b½l}$ > 2 months). In the case of the hyper-accumulator clams, whole-body loss of ^{110m}Ag was followed individually in two individuals and was best fitted by a single component exponential model, characterized by $T_{b½} \geq 2$ months.

Comparison of the radiotracer body distributions at the end of uptake (28 d) and depuration (59 d) periods indicated that the distribution remained constant for all radiotracers, except for ^{109}Cd which content in the whole soft parts increased from 40 % at the end of uptake to 86 % at the end of the depuration period (Table 3).

III.2. EXPOSURE VIA SEDIMENTS

The uptake kinetics of all sediment-bound radiotracers investigated were best fitted using a saturation model, for which the steady state was reached during the experiment time (Table 4, Fig. 3A). The estimated values of transfer factor at steady state (TF_{ss}) indicated that sediment-bound radiotracers can be ranked by order of bioavailability as follow: $^{110m}Ag > {}^{57}Co \approx {}^{65}Zn > {}^{109}Cd > {}^{51}Cr$.

After 35 d of sediment exposure, radioanalysis of dissected body compartments showed that the digestive gland bioaccumulated sediment-bound radiotracers up to one order of magnitude higher than the shells and/or the remaining soft parts (Table 5). Among the radiotracers tested, ^{51}Cr displayed the highest TF_{35d} in the whole soft parts and especially in the digestive gland (TF_{35d} = 2.5 and 12.5, respectively). Comparison of results from seawater and sediment exposures showed that the whole-body TF was lower by 1 to 4 orders of magnitude than whole-body CF calculated in the seawater experiment. In term of activity distribution at the end of uptake period (35 d), shells contained the highest proportion of ^{51}Cr and ^{57}Co (> 71 %), whereas ^{65}Zn, ^{109}Cd and ^{110m}Ag were distributed similarly between shells and whole soft parts (Table 6).

When clams were replaced in non-contaminated conditions for 49 d, whole-body loss kinetics of the five radiotracers were best fitted by a double exponential model (Table 4, Fig. 3B). Elimination of 9 to 42 % of the total radioactivity followed the short-lived component, and was characterized by short retention time ($T_{b½s} \leq 3$ d). The long-lived component represented the highest fraction of all radiotracers (A_{0l} = 58 - 91 %) which was assimilated and efficiently retained in clams ($T_{b½l} \geq 1$ month).

Table 4. Estimated uptake kinetic parameters (A) and loss kinetics parameters (B) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in whole-body *Gafrarium tumidum* exposed to radiotracers via sediments for 35 d (uptake period; n = 20) and then maintained for 49 d in non contaminated conditions (depuration period; n = 15).

Uptake parameters. TF_{ss}: transfer factors at steady state; k_u: uptake rate constant (d^{-1}).

Depuration parameters. A_{0s} and A_{0l}: activity (%) lost according to the short- and the long-lived exponential component, respectively; $T_{b½}$: biological half-life (d).

ASE: asymptotic standard error; R^2: determination coefficient of the kinetics

	A. Uptake kinetic parameters			B. Loss kinetic parameters				
Isotopes	**TF_{ss} ± ASE**	**k_u ± ASE**	**R^2**	**A_{0s} ± ASE**	**$T_{b½s}$ ± ASE**	**A_{0l} ± ASE**	**$T_{b½l}$ ± ASE**	**R^2**
^{51}Cr	0.024 ± 0.005 ^{d}	0.90 ± 0.24 ^{c}	0.47	38.7 ± 6.4 ^{d}	1.2 ± 0.5 ^{a}	61.2 ± 4.2 ^{d}	134 ± 66.0 ^{a}	0.31
^{57}Co	0.082 ± 0.001 ^{d}	0.87 ± 0.13 ^{d}	0.75	8.7 ± 1.6 ^{d}	1.1 ± 0.6 ^{a}	91.3 ± 1.0 ^{d}	85 ± 4.4 ^{d}	0.82
^{65}Zn	0.078 ± 0.002 ^{d}	0.19 ± 0.02 ^{d}	0.69	13.7 ± 3.1 ^{d}	1.4 ± 0.7 ^{a}	86.4 ± 2.1 ^{d}	74 ± 7.0 ^{d}	0.64
^{109}Cd	0.038 ± 0.001 ^{d}	0.61 ± 0.17 ^{b}	0.47	32.3 ± 5.4 ^{d}	2.7 ± 1.0 ^{b}	67.7 ± 4.5 ^{d}	127 ± 49 ^{a}	0.45
^{110m}Ag	0.107 ± 0.004 ^{d}	0.75 ± 0.29 ^{c}	0.26	42.1 ± 9.1 ^{d}	3.3 ± 1.3 ^{a}	57.6 ± 9.6 ^{d}	31 ± 6.9 ^{d}	0.80

Probability of the model adjustment: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$, * $p > 0.05$

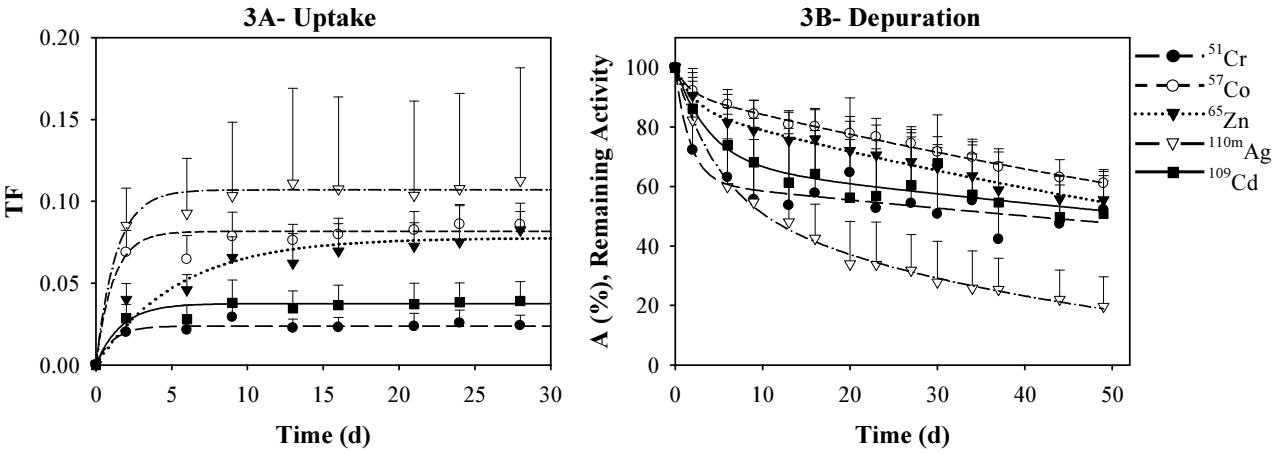
Table 5. Transfer factors (TF, mean ± SD; n = 5) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in the *Gafrarium tumidum* after 35 d of exposure via sediment.

Body compartments	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
in toto	**0.024 ± 0.008**	**0.09 ± 0.014**	**0.082 ± 0.015**	**0.042 ± 0.014**	**0.11 ± 0.07**
Shells	**0.64 ± 0.07**	**0.057 ± 0.008**	**0.031 ± 0.014**	**0.018 ± 0.009**	**0.051 ± 0.012**
Whole soft Part	**2.5± 0.83**	**0.13 ± 0.03**	**0.24 ± 0.04**	**0.20 ± 0.05**	**0.73 ± 0.97**
Digestive gland	12.9 ± 6.2	0.37 ± 0.21	0.76 ± 0.35	0.69 ± 0.36	3.76 ± 6.53
Remaining soft parts	1.4± 0.04	0.11 ± 0.03	0.19 ± 0.03	0.15 ± 0.05	0.40 ± 0.35

Table 6. Body distribution (mean % ± SD, n = 5) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* at the end of the 35-d sediment exposure (A) and at the end of the subsequent 49-d depuration period (B).

Body compartments	Weight (%)	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
A. Uptake period (35 d)						
Shells	**90.0 ± 1.1**	**70.6 ± 6.9**	**79.5 ± 5.0**	**51.2 ± 17.0**	**44.0 ± 12.7**	**53.7 ± 22.7**
Whole soft parts	**10.0 ± 1.1**	**29.4 ± 6.9**	**20.5 ± 5.0**	**48.8 ± 17.0**	**56.0 ± 12.7**	**46.3 ± 22.7**
Digestive gland	10.9 ± 4.7	50.1 ± 4	26.4 ± 6.0	36.2 ± 21.9	47.2 ± 34.8	33.9 ± 8.7
Remaining soft parts	89.1 ± 4.7	49.9 ± 4	73.6 ± 6.0	63.8 ± 21.9	52.8 ± 34.8	66.1 ± 8.7
B. Depuration period (49 d)						
Shells	**87.3 ± 1.4**	**27.6 ± 9.2**	**64.4 ± 10.9**	**43.0 ± 8.5**	**28.7 ± 16.7**	**10.5 ± 5.6**
Whole soft parts	**12.7 ± 1.4**	**72.4 ± 9.4**	**35.6 ± 10.9**	**57.0 ± 8.5**	**71.3 ± 16.7**	**89.5 ± 5.6**
Digestive gland	12.3 ± 4.5	40.4 ± 6.2	19.9 ± 6.7	18.9 ± 6.0	49.0 ± 22.4	39.5 ± 16.5
Remaining soft parts	87.7 ± 4.5	59.6 ± 6.2	80.1 ± 6.7	81.1 ± 6.0	51.0 ± 22.4	60.5 ± 16.5

Figure 3. Uptake and loss kinetics of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* (3A) exposed to radiotracers for 35 d in sediment (transfer factors, TF, mean ± SD, n = 20), and then (3B) maintained for 49 d in non contaminated conditions (% remaining activity, A, mean ± SD; n = 15).



III.3. EXPOSURE VIA THE FOOD

When clams were exposed to radiolabelled *Isochrysis galbana*, whole-body loss kinetics were best described by a double exponential model (Fig. 4), with the exception of ^{51}Cr, whose biokinetics could not be determined accurately due to detection problem linked to the low activity in clams (Table 7, Fig. 4). The short-lived component represented 21 to 64 % of the total ^{65}Zn, ^{109}Cd and ^{110m}Ag load ingested with food, which was rapidly lost during the first days of depuration ($T_{b½s} \leq 0.7$ d) (Table 7). The fraction of radiotracers associated with the long-lived component represented 36 to 79 % of ingested radiotracer load. This assimilated fraction was efficiently retained in clams ($T_{b½l} \geq 1$ month).

At the end of the depuration period (72 d), the radiotracer activity distribution among body compartments indicated that the whole soft parts contained the main fraction of all radiotracers (> 83 %). Among the considered soft tissue compartments, most of the ^{51}Cr, ^{57}Co and ^{65}Zn (20 - 32 %) was found in the mantle whereas the digestive gland contained from 40 to 42 % of ^{109}Cd and ^{110m}Ag (Table 8).

Table 7. Parameters of the loss kinetics of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* (n = 12) previously fed for 2 hrs on radiolabelled *Isochrysis galbana* cells.

A_{0s} and A_{0l}: activity (%) lost according to the short-lived (s) and the long-lived (l) exponential component, respectively (A_{0l} is the assimilation efficiency, AE); $T_{b½}$: biological half-life (d); ASE: asymptotic standard error; R^2: determination coefficient of the loss kinetics; n.d.: not determined.

Isotopes	A_{0s} ± ASE	$T_{b½s}$ ± ASE	AE ± ASE	$T_{b½l}$ ± ASE	R^2
^{51}Cr	n.d.	n.d.	n.d.	n.d.	n.d.
^{57}Co	21.1 ± 3.7 ^{d}	0.4 ± 0.3 *	78.8 ± 2.0 ^{d}	52.4 ± 5.3 ^{d}	0.64
^{65}Zn	64.0 ± 2.8 ^{d}	0.4 ± 0.1 ^{d}	35.9 ± 1.6 ^{d}	38.0 ± 5.4 ^{d}	0.85
^{109}Cd	58.3 ± 5.3 ^{d}	0.2 ± 0.1 *	41.7 ± 2.5 ^{d}	66.4 ± 19.1 ^{c}	0.53
^{110m}Ag	50.8 ± 5.2 ^{d}	0.7 ± 0.2 ^{c}	48.8 ± 3.7 ^{d}	28.4 ± 5.2 ^{d}	0.69

Probability of the model adjustment: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$, * $p > 0.05$

Figure 4. Loss kinetics of ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* after a 2-hr single feeding on radiolabelled *Isochrysis galbana* cells (% remaining activity, A, mean ± SD; n = 12).

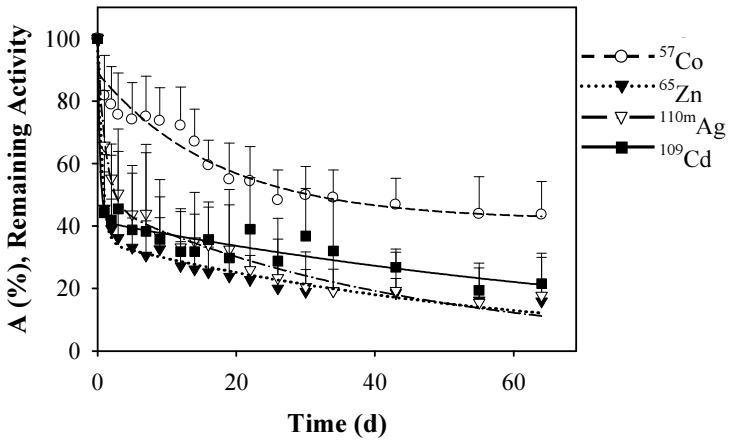


Table 8. Body distribution (mean % ± SD, n = 3) of ^{51}Cr, ^{57}Co, ^{65}Zn, ^{109}Cd and ^{110m}Ag in *Gafrarium tumidum* 72 d after a 2-hr single feeding on radiolabelled *Isochrysis galbana* cells.

Body compartments	Weight (%)	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag
Shells	**90.2 ± 0.6**	**16.8 ± 4.4**	**14.6 ± 3.8**	**10.4 ± 3.3**	**9.8 ± 6.8**	**6.9 ± 4.3**
Whole soft parts	**9.8 ± 0.6**	**83.2 ± 4.4**	**85.4 ± 3.8**	**89.6 ± 3.3**	**90.2 ± 6.8**	**93.1 ± 4.3**
Gills	17.8 ± 4.1	18.8 ± 3.2	15.2 ± 5.9	13.9 ± 3.4	12.2 ± 12.0	6.7 ± 4.3
Adductor muscle	17.7 ± 4.0	13.2 ± 3.3	17.3 ± 5.5	11.7 ± 4.1	11.5 ± 8.5	7.6 ± 5.2
Mantle	23.0 ± 1.7	20.0 ± 3.8	22.9 ± 5.3	32.0 ± 20.0	13.8 ± 6.8	26.0 ± 16.9
Digestive gland	12.5 ± 6.1	21.1 ± 5.6	17.2 ± 4.0	18.8 ± 9.8	42.0 ± 27.2	40.3 ± 25.7
Foot	12.8 ± 3.4	12.3 ± 8.7	5.9 ± 7.5	8.7 ± 10.1	3.8 ± 6.2	3.2 ± 4.7
Remaining soft parts	16.2 ± 5.7	14.6 ± 1.3	21.3 ± 6.0	14.8 ± 11.7	16.7 ± 6.9	16.3 ± 12.0

IV. DISCUSSION

In the marine environment, filter-feeder organisms are exposed to metals via dissolved and/or particulate pathways. Consequently, in order to determine the bioindicative potential of the clam *Gafrarium tumidum*, it was necessary to assess the bioaccumulation and retention capacities of this bivalve following exposures to metals via different pathways (seawater, sediment and food).

After the exposure to dissolved metal radiotracers for a relatively long period of time (28 d), the activities measured in clam tissues were 33 (Cr) to 4982 (Ag) fold higher than the waterbone radioactivity. This indicates that the clam displayed efficient capacities for bioconcentrating dissolved metals, and thus, an interesting potential as bioindicator of dissolved contamination. However, the high bioconcentration of Ag was quite surprising due to its recognized toxicity for aquatic organisms (Ratte 1999). Such a high bioconcentration potential compared to the others metals suggests the occurrence of a specific detoxification

process for Ag in clam tissues In addition, four individuals have been identified as hyper-accumulator for this metal, showing CF one to two orders of magnitude higher than the other clams. It is well documented that bivalves can store large amounts of Ag as Ag_2S in their tissues, which is a very stable amorphous material generally found precipitated in the basement membranes and underlying connective tissues (e.g. Berthet et al. 1992). The relatively long retention time of Ag ($T_{b½}$ > 2 months) strongly suggests that such a protecting mechanism occurs in *G. tumidum*. Moreover, previous results from the subcellular distribution in the clam tissues also support this hypothesis. Indeed, after a 28-d exposure to dissolved Ag, this metal was predominantly distributed in the insoluble fraction (viz. organelle and membrane fraction) of gills (73 %) and visceral mass (49 %) of the clam *G. tumidum* (Metian et al. 2005).

Although historically seawater pathway has been considered as the main source of metal bioaccumulation in marine organisms, food and particulate pathways have received an increasing attention this last decade and were shown to contribute importantly (sometimes predominantly) to global bioaccumulation in marine organisms (e.g., Wang et al. 1996; Reinfelder et al. 1998).

After exposure of the clam *Gafrarium tumidum* to radiolabelled *I. galbana* cells, 35 to 79 % of the metals ingested with food were actually absorbed by the clams and strongly retained within their tissues ($T_{b½}$ > 1 month). Assimilation efficiencies -AE- estimated for *G. tumidum* were comparable to available data on marine bivalves in tropical and subtropical areas (e.g. *Ruditapes philippinarum*, Ng & Wang 2004) or in temperate waters (*Macoma balthica*, Griscom et al. 2002b; *Mercenaria mercenaria,* Reinfelder et al. 1997). Although a direct relationship between AE and the metal fraction in the phytoplankton cytoplasm has been observed for copepods and bivalve larvae (Reinfelder & Fisher 1991; Reinfelder & Fisher 1994), similar relationship was not always found for marine bivalves (e.g. Cr and Zn in the mussel *Perna viridis* and the clam *Ruditapes philippinarum,* Chong & Wang 2000a). In our experiments, the subcellular distribution of metals in *I. galbana* was not investigated. However, comparing the values reported by Reinfelder et al. (1997) for *I. galbana* (80 % of Zn in the cytoplasm) and the AE value observed here for Zn in the clam (AE = 36 %, viz. the lowest values among the 5 elements examined) suggest that the cytoplasmic distribution would not to be the only mechanism controlling AE of metals in *G. tumidum*.

For marine organisms living intimately associated with the sediments and ingesting metal-rich particles from both the water column and the seawater-sediment interface, sediments could be

an important pathway of contamination, mainly due to their metal concentration several orders higher of magnitude than those reported in seawater (Luoma 1989). Such a situation obviously corresponds to living habits of *G. tumidum*. In this work, the clam bioaccumulated sediment-bound metals up to a quite low level (TF_{35d} in whole soft parts ≤ 0.7 for all radiotracers except ^{51}Cr, $TF_{35d} = 2.5$), suggesting that sediment-bound metals are not highly transferred to the organisms. Nevertheless, Cr displayed an interesting behaviour: not only Cr was the metal displaying the highest TF in clam tissues, but sediment-bound Cr was also efficiently absorbed in clam tissues (A_{0l} = 61%). This result is quite surprising since Cr generally display low absorption/assimilation efficiency from sediment (AE $\leq$ 1.3 %, Wang & Fisher 1996a; Wang et al. 1997) and food pathways (AE $\leq$ 10 %, Wang et al. 1997; Wang & Fisher 1999a). Interestingly, similar AE of Cr as those reported in this work has been reported for the bivalve *Potamocorbula amurensis* (67%) fed on ^{51}Cr-labelled bacteria (Decho & Luoma 1991). The reduction of Cr (VI) to Cr (III) by bacteria has been demonstrated in several studies (e.g. Cheung & Gu 2003), and bacteria could thus modify the bioavailability of Cr for organisms. Such a mechanism leading to an enhanced bioavailability of Cr could be hypothesized for *G. tumidum* which is known to feed at least partly on particles from sediment-water interface which are particularly rich in bacteria.

This study clearly pointed out that *G. tumidum* displayed a particular pattern of bioaccumulation of sediment-bound metals. Indeed, absorption efficiencies of sediment-bound metals (from 58 to 91 %) were always higher than those estimated for metals ingested with food (from 36 to 79 %), and were generally higher than available data reported in literature (e.g. Wang & Fisher 1999a; Griscom et al. 2000; Griscom et al. 2002a). The most common trend in marine organisms is to display a higher AE for metals ingested with food than for sediment-bound metals (Lee & Luoma 1998; Chong & Wang 2000b). Such a high absorption efficiency of sediment-bound metals could be due to a more efficient desorption of sediment-bound metals than phytoplankton-bound metals in the acidic environment of the gut of *G. tumidum* or to a simultaneous absorption of metals sediment porewater or overlying water (Mayer et al. 1996), in which highly desorbed metals are available for diffusion across the gut lining. Although the mechanisms through which sediment-dwelling organisms take up metals from the overlying water compartment or the sediment compartment or both is not well understood (Luoma 1989; Hansen et al. 1996; Selck et al. 1998; Warren et al. 1998), there are known to be species-dependent. Hence, the high absorption of sediment-bound metals observed here would deserve further investigations.

The TF in clam tissues estimated from sediment exposure were lower by 1 to 4 orders of magnitude than CF calculated from seawater exposure, suggesting that metals immobilized in sediments are much less bioavailable for *G. tumidum* than dissolved metals. Similar results have been already observed in the oysters *Isognomon isognomon* and *Malleus regula* from the New Caledonia lagoon (Hédouin et al. to be submitted-b). Nevertheless, this does not imply that sediments could not be an important pathway of accumulation. Indeed, since metal concentrations reported in sediments are generally several orders of magnitude higher than those measured in seawater, the importance of sediments vs seawater bioaccumulation pathways in clams should be investigated in terms of relative contribution taking into account the distribution of metals between seawater and sediments. Therefore, further studies should be carried out in order to allow for quantifying the relative importance of sedimentary, dietary and dissolved pathways in the global bioaccumulation of metals in *G. tumidum*, using bioaccumulation models (e.g. Thomann et al. 1995; Wang et al. 1996).

Once accumulated, metals were efficiently retained in clam tissues ($T_{b½}$ > 1month) whatever the metal and the uptake pathway considered. Whereas sediment-bound metals and dissolved metals displayed very similar depuration kinetics and close $T_{b½}$, metals ingested with food were less efficiently retained, indicating that the exposure pathway may influence processes of metal storage in the clam tissues. If verified, such an hypothesis would have important implications regarding the clam metal fraction metabolically available for consumers (e.g., Man) (Rainbow 1996; Ettajani et al. 2001). For example, a previous study on sub-cellular distribution of metals in the clam *G. tumidum* showed that Ag was mainly present in the insoluble fraction and hence suggested that is was slightly bioavailable for the higher trophic levels whereas Cd, Co, Cr and Zn, being mainly distributed in the cytosolic fraction, would be highly bioavailable to the consumers (Metian et al. 2005). Since this study only considered seawater exposure of the clam, further studies considering food and sediment pathways could determine whether the uptake pathway influences the way metals are stored in clam tissues, and the consequences for metal bioavailability to human consumers.

V. CONCLUSION

The high bioaccumulation and retention capacities for Ag, Cd, Co, Cr and Zn of the clam *G. tumidum* indicated that this species would be able to inform fastly about contamination events (high values of k_u) as well as to preserve the information for a long period of time ($T_{b1/2} \geq 1$

month). Ag being known as a reliable proxy for domestic sewage inputs (Sanudo-Willhelmy & Flegal 1992), the very efficient bioaccumulation of Ag in clam tissues indicates that it could be used as a bioindicator of domestic contamination in parallel to a bioindicator of mining-originating contamination.

It is concluded that the implementation of a programme to survey and monitor the quality of the lagoon waters in New Caledonia should consider the use the clam *Gafrarium tumidum*. Besides its potential as a proxy for mining and domestic contamination, it would also provide useful health information as it is a seafood commonly consumed by the local populations.

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CHAPITRE 4

Bioaccumulation du Ni dans des bivalves tropicaux du lagon de Nouvelle-calédonie : Exposition via l'eau de mer et la nourriture

Les huîtres *Isognomon isognomon* et *Malleus regula* et le clam *Gafrarium tumidum* ont été exposés au Ni via l'eau et la nourriture en utilisant les techniques de radiotraçage. Les résultats montrent que l'efficacité d'accumulation et de rétention du Ni sont indépendants des concentrations en Ni dissout. Ainsi, pour les trois espèces, les concentrations en Ni accumulé à partir de la phase dissoute sont directement proportionnelles aux concentrations dissoutes ambiantes. Les cinétiques de dépuration indiquent que la majeure partie du Ni est rapidement éliminée par les bivalves pendant les premiers jours de perte, alors que 7 à 47 % du ^{63}Ni sont retenus efficacement dans les tissus avec un temps de demie-vie non significativement différent de l'infini. Enfin, les expériences d'alimentation montrent que le Ni ingéré avec la nourriture (phytoplancton) est assimilé plus efficacement dans les clams (efficacité d'assimilation, EA = 61 %) que dans les huîtres (EA = 17%), et fortement retenu ($T_{b½} \geq 35$ j) dans les tissus des deux bivalves. Ainsi, les espèces étudiées sont des bioaccumulateurs efficaces du Ni à partir de l'eau ambiante et de la nourriture, et elles pourraient constituer des bioindicateurs fiables pour la surveillance du degré de contamination en Ni dans les eaux côtières tropicales.

Nickel bioaccumulation in bivalves from the New Caledonia lagoon: Seawater and food exposure

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ABSTRACT. The tropical oysters *Isognomon isognomon* and *Malleus regula* and the clam *Gafrarium tumidum* were exposed to Ni via seawater or food using radiotracer techniques. Results indicate that uptake and retention efficiencies of Ni are independent on the Ni dissolved concentrations in the surrounding seawater. Hence, for the three species, body concentrations of Ni taken up from dissolved phase are directly proportional to the ambient dissolved concentrations. Depuration kinetics indicated that the major part of Ni was rapidly lost from bivalves during the first days of depuration, whereas 7 to 47 % of ^{63}Ni were retained in tissues with a biological half-life not significantly different from infinite. Finally, feeding experiments showed that Ni ingested with food (phytoplankton) was assimilated more efficiently in clams (assimilation efficiency, AE = 61 %) than in oysters (AE = 17 %) and strongly retained ($T_{b½} \geq 35$ d) in the tissues of both bivalve groups. It is concluded that the investigated species are efficient bioaccumulator of Ni from both the surrounding seawater and the food and that they would be valuable bioindicators for monitoring Ni contamination status in tropical coastal waters.

Keywords: Metal, Molluscs, Mining Activities, Bioindicator, Radiotracer

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I. INTRODUCTION

Besides its status of second largest lagoon in the world, a high biodiversity and a high endemism rate, the New Caledonian lagoon is also subject to high contamination pressure (Labrosse et al. 2000; Bouchet et al. 2002). Indeed, the largest laterite Ni resources in the world (20-25 %) are present in New Caledonia, which is currently the third world producer of Ni (Dalvi et al. 2004). Hence, mining activities are the major economic resources of New Caledonia.

Laterite deposits are made up of two ore sources available for mining extraction: limonite and saprolite ores, containing 1 to 1.6 % and 1.6 to 3 % of Ni, respectively. Due to their higher Ni content, saprolite ores have been traditionally exploited since the end of the nineteenth century using pyrometallurgical process. Since the future trend is expected to use limonite ores as well; INCO, one of the world largest Ni producers, is currently developing a hydrometallurgical extraction plant at Goro in the southern territory of New Caledonia, based on acidic extraction (viz. lixiviation).

Mining activities and their development represent an issue of concern in New Caledonia, due to increasing deforestation, soil erosion, extinction of endemic species and increasing Ni water contamination (Bird et al. 1984; Labrosse et al. 2000). However, information available on impacts of open-cast mining in marine coastal ecosystems of New Caledonia is extremely scarce (Monniot et al. 1994; Breau 2003; Hédouin et al. in press).

Among the common approaches used to survey environmental contamination, the use of bioindicator species has been proved to be a valuable and informative tool (Goldberg et al. 1983; Phillips 1990). In order to develop a biomonitoring programme to assess metal contamination levels in New Caledonia lagoon, studies have been recently undertaken to screen local species that could be used as bioindicators (Breau 2003). In particular, both laboratory and field studies have shown that two oysters (*Isognomon isognomon* and *Malleus regula*) and one clam (*Gafrarium tumidum*) concentrate efficiently several elements (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn) and that they are able to discriminate locations subject to contrasted levels of contamination (Hédouin et al. submitted-a). However, currently available information regarding Ni bioaccumulation is rather limited; in particular no data is available on body distribution or bioaccumulation kinetics of Ni from dissolved and dietary pathways in these organisms. Therefore, the objective of the present study was to determine the

bioaccumulation capacity of Ni from food and seawater in the three above mentioned bivalves in order to assess their usefulness as bioindicators of Ni contamination.

II. MATERIALS & METHODS

II.1. COLLECTION AND ACCLIMATION

The clams *Gafrarium tumidum* were collected by sea-shore fishing in Dumbéa Bay and the oysters *Isognomon isognomon* and *Malleus regula* were collected by SCUBA diving in October 2003 in Maa Bay in the same environment, Nouméa, New Caledonia. Despite a considerable sampling effort, only a limited number of both oyster species have been collected compared to the number requested for planned experiments. Nevertheless, as the oysters *I. isognomon* and *M. regula* are extremely close species both from their appearance and life mode viewpoints (Yonge 1968), *M. regula* was used as oyster representative in the seawater exposure experiments, whereas *I. isognomon* was used as oyster representative in the food exposure experiments. To ensure comparability of both oysters, some *I. isognomon* individuals were also included in the seawater experiments.

Oysters and clams were acclimated to laboratory conditions for one week prior to the experiments (close circuit aquarium; daily water renewal, salinity: 35 ± 1 p.s.u.; temperature = 25 ± 1°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). Since body size is known to affect metal concentrations in organisms (Boyden 1977), only individuals with shell width longer than 35 mm for *G. tumidum* and shell length longer than 70 mm for *I. isognomon* and *M. regula* were considered for the experiments (Metian 2003; Hédouin et al. in press).

II.2. TESTING INFLUENCE OF DISSOLVED NI CONCENTRATIONS

Five groups of 51 clams *G. tumidum* (shell width from 35 to 44 mm; wet wt from 15 to 38 g); five groups of 51 oysters *M. regula* (shell length from 85 to 135 mm; wet wt from 14 to 48 g) and five groups of 5 oysters *I. isognomon* (shell length from 80 to 140 mm; wet wt from 13 to 45 g) were dispatched in 5 aquaria of 50 l of natural seawater (close circuit aquarium; salinity: 35 ± 1 p.s.u.; temperature = 25 ± 1°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). Seawater salinity, temperature and pH were checked twice daily along the experiment duration.

Each group of bivalves was exposed for 14 d to five increasing added Ni concentrations (0, 15, 75, 350 and 1400 ng Ni l^{-1}). Added concentrations of Ni were realized using increasing amounts of $Ni(NO_3)_2$ (Merck, synthesis quality) and a fixed activity (1 kBq l^{-1}) of the corresponding radiotracer ^{63}Ni, as high specific activity $^{63}NiCl_2$ ($T_{½}$ = 100 yrs) purchased from Amersham, UK. This radiotracer spike corresponded to 4.2 ng stable Ni l^{-1}, a concentration at least 1 order of magnitude lower than the background concentrations of Ni in open seas (Bruland 1983). No change in pH was detectable after stable metal and radiotracer addition. Seawater and spikes were renewed daily for 5 d, then every second day in order to keep exposure concentration and activity as constant as possible. Activity of the radiotracer in seawater was checked daily, and before and after each seawater renewal to determine its time-integrated activity (Warnau et al. 1996b). For the entire experimental time course, the time-integrated ^{63}Ni activity in seawater was 0.81 kBq l^{-1}.

During the exposure period, 3 individuals of *G. tumidum* and *M. regula* were collected at different time intervals; soft tissues were separated from shells and prepared for whole soft tissues radioanalysis (see section II.4.). The last day (t_{14d}), 5 individuals of *G. tumidum, M. regula* and *I. isognomon* were dissected to determine body distribution of incorporated ^{63}Ni. Dissected body compartments were digestive gland, gills, mantle, foot, muscle and remaining soft tissues for clams and visceral mass + mantle, gills and muscle for oysters.

At the end of the 14-d exposure, the remaining organisms were placed in 60 x 60 x 60 cm plastic cages for 32 d in Sainte-Marie Bay, Nouméa, New Caledonia at 7 m depth. The selected area (22°18'55 S, 166°27'98 E) is characterized by relatively low Ni levels (Hédouin et al. submitted-a). At different time intervals of the depuration period, for each concentration tested, 3 *G. tumidum* and 3 *M. regula* individuals were collected, dissected to separate soft tissues from shells and prepared for soft parts radioanalysis. The last day (t_{32d}), 5 *G. tumidum* were dissected in their different body compartments to determine body distribution of remaining ^{63}Ni.

II.3. EXPOSURE VIA THE FOOD

Cells of the Prymnesiophyceae *Isochrysis galbana* (10^3 cell ml^{-1}) from axenic stock cultures were resuspended in an erlenmeyer flask containing 5 l sterile-filtered (0.22 µm) seawater enriched with f/2 nutrients without EDTA and Si. Seawater was spiked with ^{63}Ni (5 kBq l^{-1}), and the cells were then incubated at 25°C (light/dark cycle: 12 hrs/12 hrs). After 6 d of incubation, cell density increased from 10^3 to 1.3 10^6 cell ml^{-1}. A sample of 10 ml of the

culture was then gently filtered (47 mm diameter Polycarbonate Nuclepore® filter, 1 µm mesh size) and the radioactivity associated with *I. galbana* cells was mesured before and after the filtration (3.4 10^{-6} Bq ^{63}Ni $cell^{-1}$).

Bivalves (n = 196 *G. tumidum,* n = 196 *I. isognomon)* were placed in a 300 l aquarium (close circuit aquarium constantly aerated; salinity: 35 ± 1 p.s.u.; temperature = 25 ± 1°C; pH = 8.0 ± 0.1) and fed the radiolabelled *I. galbana* for 2 hrs (10^4 cell ml^{-1}). Immediately after feeding, 14 individuals per species were collected and dissected to separate whole soft parts from shells.

The remaining bivalves were then placed in Sainte-Marie Bay in plastic cages as previously described. At different time intervals, 14 individuals of each species were collected in order to follow loss kinetics of ^{63}Ni ingested with food. At 12 and 46 d, collected individuals were dissected to determine the distribution of ^{63}Ni contents among the different body compartments.

II.4. SAMPLE PREPARATION AND RADIOANALYSES

Seawater samples (2 ml) were directly transferred to 20-ml glass scintillation vials (Packard) and mixed with 10 ml of scintillation liquid (Ultima Gold®, Packard). The separated body compartments and whole soft parts of bivalves were weighed (wet wt), dried at 60°C until constant weight, and weighed again (dry wt). Clam and oyster tissues were then digested for one week (50°C) with 1 ml Soluene® (Packard) per 100 mg dry wt tissues, and then mixed with scintillation liquid (Hionic Fluor®, Packard) in proportion 1: 5 (v: v). Bivalve shells were leached in three successive baths (20 min) of HCl 2N in order to recover all the ^{63}Ni adsorbed onto shells. Samples of 1 ml were transferred to 20-ml glass scintillation vials and mixed in proportion 1:10 (v: v) with scintillation liquid (Ultima Gold®).

The radioactivity of ^{63}Ni was counted using a 1600 TR Packard Liquid Scintillation Analyzer. Counting time was adapted to obtain a propagated counting error less than 5 % (maximal counting duration 2 hrs). The radioactivity was determined by comparison with standards of known activities and measurements were corrected for counting efficiency, physical radioactive decay and quenching effects.

II.5. DATA ANALYSES

Uptake of ^{63}Ni was expressed in terms of concentration factor (CF: ratio between activity of the radiotracer in the whole soft parts or in a body compartment -Bq g^{-1} dry wt- and time-

integrated activity of the radiotracer in seawater -Bq ml^{-1}-). Radiotracer uptake kinetics were described using either a simple linear regression model (eq. 1) or, if the observed kinetics tended to reach a steady state, a saturation exponential model (eq. 2):

$CF_t = k_u\, t$ (eq. 1)

$CF_t = CF_{ss}\,(1- e^{-k_e\, t})$ (eq. 2)

where CF_t and CF_{ss} are the concentration factors at time t (d) and at steady state (ml g^{-1}), ($CF_{ss} = k_u/k_e$); k_u is the uptake rate constant (ml g^{-1} d^{-1}) and k_e is the depuration rate constant (d^{-1}) (Whicker & Schultz 1982; Thomann et al. 1995). Linearity of the uptake kinetics was tested by a linearity test for regression with replication (Zar 1996).

Loss of ^{63}Ni was expressed in term of percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in the organisms immediately after the feeding period). The loss kinetics were best fitted by either a single-component exponential equation (eq. 3), a single-component exponential equation with an additional constant term (eq. 4), or a double-component exponential equation (eq. 5):

$A_t = A_0\, k_e\, t$ (eq. 3)

$A_t = A_{0s}\, e^{-k_{es}\, t} + A_{0l}$ (eq. 4)

$A_t = A_{0s}\, e^{-k_{es}\, t} + A_{0l}\, e^{-k_{el}\, t}$ (eq. 5)

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components. For each exponential component (s and l), a biological half-life can be calculated ($T_{b½s}$ and $T_{b½l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b½} = \ln 2/k_e$. The additional constant term of equation 4 represents a fraction A_{0l} of the radiotracer incorporated that is virtually sequestrated in the organism tissues.

Regarding feeding experiments, the 'long-lived' exponential term describes the proportion of the radiotracer ingested with food that is actually absorbed by the organism and slowly eliminated. The corresponding A_{0l} represents the assimilation efficiency (AE) of the considered element.

Model constants and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation, respectively using the nonlinear curve-fitting routines in the

Statistica 5.2.1 software. Best fitting models were selected according to the highest determination coefficient and examination of residuals.

In order to assess possible effect of dissolved Ni concentration on bioconcentration behaviour, estimated kinetic parameters (k_u, CF_{ss}, A_{0l}, k_{el}) were plotted against the concentration of total Ni (stable + stable equivalent of added radiotracer) in seawater and were fitted using simple linear regression. Statistical comparisons were also performed using 1-way ANOVA followed by the multiple comparison test of Tukey (Zar 1996).

The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

III.1. SEAWATER EXPOSURE TO INCREASING NI CONCENTRATIONS

Uptake of ^{63}Ni in whole soft parts of the clam *G. tumidum* and the oyster *M. regula* displayed linear kinetics at all of the five exposure concentrations tested ($p < 0.0001$, R^2: 0.79-0.92 for *G. tumidum* and 0.63-0.72 for *M. regula*) (Table 1 and Fig. 1-A1). Statistical analysis indicated that uptake rate constants k_u in both clams and oysters did not differ significantly over the range of concentrations tested.

After 14 d of exposure, the concentration factors (CF_{14d}) of ^{63}Ni were calculated in the different body compartments of the clam as well as in both oysters (*M. regula* and *I. isognomon*) (Table 2). In all three bivalves, whole soft part CF_{14d} was one to three orders of magnitude higher than those calculated for shells. Table 2 indicates that ^{63}Ni was concentrated selectively by the different body compartments in each species, according to the following order:

- *G. tumidum*: digestive gland (CF_{14d} up to 620) > gills > remaining tissues > mantle > muscle ≈ foot,

- *I. isognomon*: gills (CF_{14d} up to 660) > visceral mass + mantle > muscle,

- *M. regula*: visceral mass + mantle (CF_{14d} up to 265) > gills ≈ muscle.

In general, no significant difference was found among CF_{14d} in whole soft parts as well as in body compartments over the range of concentrations tested. The only exception was *I. isognomon*, for which the CF_{14d} calculated in whole soft parts, gills and visceral mass +

mantle for the highest Ni concentration (1400 ng added Ni l^{-1}) were found to be significantly different from the ones calculated for 75 ng added Ni l^{-1} (p_{Tukey} = 0.026, 0.046 and 0.043, respectively).

Table 1. Estimated uptake rate constant (k_u, ml g^{-1} dry wt d^{-1}), absorption efficiency (A_{0l}, %) and loss rate constant (k_{el}, d^{-1}) of ^{63}Ni in the whole soft parts of the clam *Gafrarium tumidum* and the oyster *Malleus regula* exposed to five increasing dissolved Ni concentrations via seawater for 14 d (uptake period) and then maintained for 32 d in the field in a clean site (depuration period).

ASE: asymptotic standard error; R^2: determination coefficient of the uptake and loss kinetics

Concentrations (ng added Ni l^{-1})	Uptake period		Depuration period		
	$k_u \pm$ ASE	R^2	$A_{0l} \pm$ ASE	$k_{el} \pm$ ASE	R^2
G. tumidum					
C_0: 0	9.1 ± 0.6 [c]	0.79	44 ± 11 [b]	0.010 ± 0.013*	0.72
C_1: 15	7.2 ± 0.4 [c]	0.83	27 ± 20*	0.002 ± 0.028*	0.84
C_2: 75	7.7 ± 0.4 [c]	0.82	45 ± 18 [a]	0.002 ± 0.017*	0.60
C_3: 350	7.1 ± 0.4 [c]	0.82	47 ± 7 [c]	0.019 ± 0.009*	0.70
C_4: 1400	5.2 ± 0.2 [c]	0.92	35 ± 3 [c]	0.001 ± 0.006*	0.77
M. regula					
C_0: 0	11.5 ± 1.0 [c]	0.72	14 ± 2.3 [c]	#	0.90
C_1: 15	10.1 ± 0.8 [c]	0.68	7 ± 3.6 [a]	#	0.90
C_2: 75	14.0 ± 1.3 [c]	0.67	13 ± 2.6 [c]	#	0.90
C_3: 350	10.7 ± 1.0 [c]	0.63	11 ± 2.1 [c]	#	0.94
C_4: 1400	8.1 ± 0.7 [c]	0.70	9 ± 5.5*	#	0.78

According to the equation fitting the loss kinetics ($A_t = A_{0s}\, e^{-k_{es}\, t} + A_{0l}$) this parameter = 0

Significance of the estimated parameters: [a] $p < 0.05$, [b] $p < 0.001$, [c] $p < 0.0001$, * $p > 0.05$

Figure 1. Uptake and loss kinetics of ^{63}Ni in whole soft parts of the investigated bivalves.

(A) Uptake kinetics (mean concentration factor, CF ± SD, n = 3) (A-1) and loss kinetics (mean % remaining activity ± SD, n = 3) (A-2) in the clam *Gafrarium tumidum* and the oyster *Malleus regula* exposed to 5 increasing dissolved Ni concentrations.

(B) Loss kinetics (mean % remaining activity ± SD, n = 14) in the clam *G. tumidum* and the oyster *Isognomon isognomon* after a 2-hr feeding on ^{63}Ni-labelled *Isochrysis galbana*.

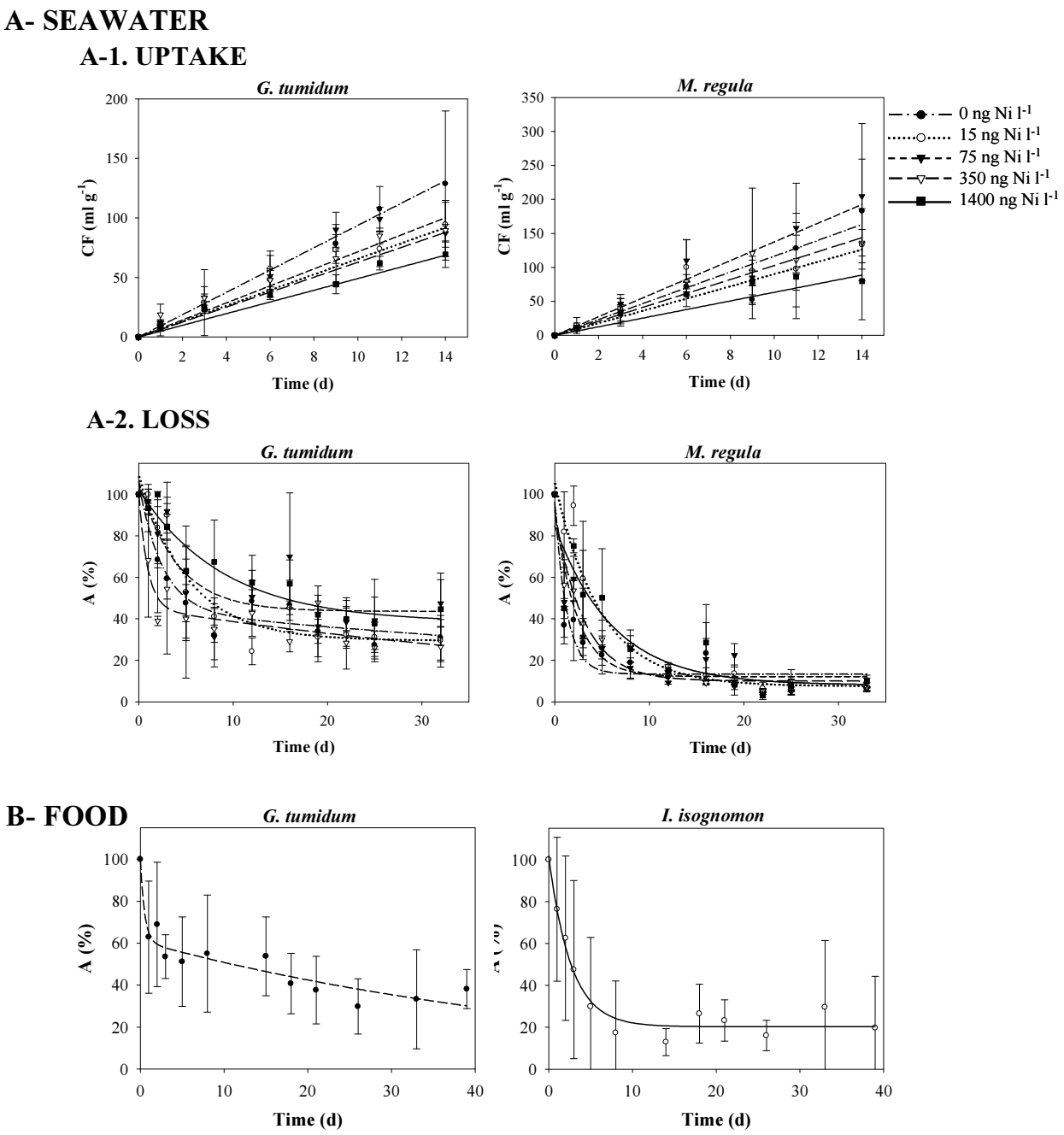


Table 2. Concentration factors (mean CF ± SD, ml g^{-1} dry wt; n = 5 per species per concentrations tested) of ^{63}Ni in the clam *Gafrarium tumidum* and the oysters *Isognomon isognomon* and *Malleus regula* exposed for 14 d to 5 increasing dissolved Ni concentrations (C_0-C_4).

Body compartments	C_0 mean ± SD	C_1 mean ± SD	C_2 mean ± SD	C_3 mean ± SD	C_4 mean ± SD
G. tumidum					
Shell	**2.0 ± 0.4**	**2.0 ± 0.3**	**2.2 ± 0.1**	**2.0 ± 0.2**	**1.6 ± 0.3**
Whole soft parts	**129 ± 61**	**94 ± 19**	**87 ± 7.3**	**90 ± 25**	**70 ± 11**
Digestive gland	620 ± 200	306 ± 126	286 ± 31	356 ± 43	235 ± 129
Gills	206 ± 11	151 ± 43	176 ± 41	164 ± 12	112 ± 24
Mantle	114 ± 52	69 ± 13	62 ± 9.4	75 ± 47	58 ± 7.1
Muscle	52 ± 18	36 ± 3.2	32 ± 8.8	42 ± 33	32 ± 3.1
Foot	17 ± 11	27 ± 6.4	29 ± 13	18 ± 4.2	16 ± 2.1
Remaining tissues	123 ± 50	96 ± 34	62 ± 11	93 ± 48	64 ± 20
I. isognomon					
Shell	**1.4 ± 0.1**	**1.6 ± 0.4**	**1.2 ± 0.4**	**1.2 ± 0.2**	**0.9 ± 0.1**
Whole soft parts	**213 ± 70**	**262 ± 20**	**284 ± 105**	**171 ± 61**	**143 ± 52**
Visceral mass + Mantle	232 ± 74	295 ± 35	305 ± 116	175 ± 71	155 ± 70
Gills	456 ± 177	519 ± 93	660 ± 265	460 ± 148	338 ± 91
Muscle	110 ± 8.3	77 ± 30	85 ± 34	113 ± 19	75 ± 47
M. regula					
Shell	**42 ± 29**	**47 ± 35**	**53 ± 36**	**60 ± 48**	**67 ± 24**
Whole soft parts	**183 ± 76**	**133 ± 53**	**205 ± 107**	**136 ± 19**	**106 ± 24**
Visceral mass + Mantle	249 ± 98	1901 ± 93	265 ± 161	186 ± 12	178 ± 43
Gills	176 ± 134	77 ± 15	94 ± 12	79 ± 13	38 ± 15
Muscle	69 ± 63	54 ± 23	101 ± 61	67 ± 48	43 ± 28

Comparisons of CF_{14d} in the whole soft parts and body compartments between *I. isognomon* and *M. regula* indicated that no significant difference was found for the whole soft parts, except for 15 ng added Ni l^{-1}, for which *I. isognomon* displayed a significantly higher CF than *M. regula* ($p_{Tukey} = 0.002$). Regarding body compartments, no significant difference was found for visceral mass + mantle and muscle between the two species (p_{Tukey} always > 0.05), whereas CF_{14d} in gills of *I. isognomon* were significantly higher than those of *M. regula* at each concentration tested (p_{Tukey} always ≤ 0.04).

In terms of body load distribution, ^{63}Ni was mainly found in the digestive gland for clams (36 to 47 % of total body load; Fig. 2-A1) and in the visceral mass + mantle for both oysters (67 to 82 % of total body load; Fig. 2-A2). Body distribution of ^{63}Ni was similar over the whole range of concentrations tested.

At the end of exposure time, non-contaminating conditions were restored and loss kinetics of ^{63}Ni were followed in the field for 32 d. Loss kinetics from whole soft parts were best described by a double exponential model in *G. tumidum*, whereas a single-component exponential equation with an additional constant term best fitted the loss kinetics in *M. regula* (Table 1 and Fig. 1-A2).

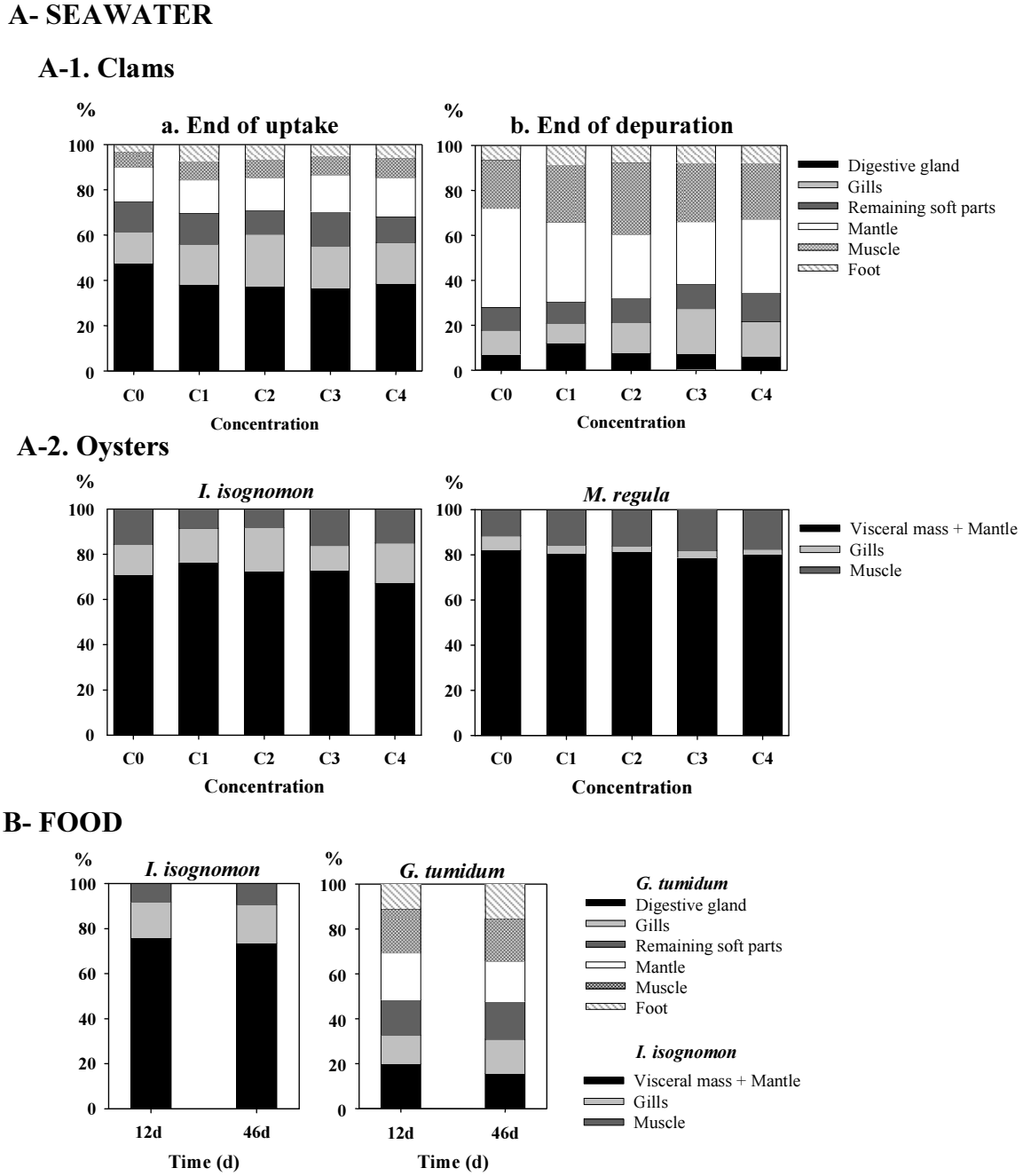
A relatively small fraction (< 14 %) of ^{63}Ni was lost according to the long-lived component in *M. regula*, whereas this component concerned 27 to 47 % of ^{63}Ni in *G. tumidum* (Table 1). However, in both species, the estimated loss rate constants of the long-lived components (k_{el}) were not significantly different from 0 ($p > 0.05$), and derived biological half lives of ^{63}Ni in clams and oysters were thus infinite whatever the exposure concentration tested. In addition, in both species, linear regressions established between estimated A_{0l} and exposure concentrations displayed slopes not significantly different from 0 for both clams ($p = 0.71$) and oysters ($p = 0.34$), indicating that ^{63}Ni was assimilated similarly (in relative %) in each species regardless the exposure concentration.

The distribution of ^{63}Ni among the body compartments of the clam was determined at the end of the depuration period (Fig. 2-A1). ^{63}Ni was mainly associated with the mantle (27 to 44 %) and the muscle (22 to 32 %). Distributions were similar for the different exposure treatments, but differed from the distributions observed at the end of the exposure period, with lower fraction associated with digestive gland and higher fractions associated with mantle and muscle.

Figure 2. Distribution of ^{63}Ni (mean %) among the body compartments of clams and oysters.

(A) Body distribution (n = 5) in the seawater experiments in (A-1) the clam *Gafrarium tumidum* after a 14-d exposure to 5 increasing dissolved Ni concentrations (end of uptake) and a subsequent 32-d depuration period (end of depuration), and (A-2) the oysters *Isognomon isognomon* and *Malleus regula* after a 14-d exposure to the 5 dissolved Ni concentrations.

(B) Body distribution (n = 14) in the clam *G. tumidum* and the oyster *I. isognomon,* 12 and 46 d after a 2-hr feeding on ^{3}Ni-labelled *Isochrysis galbana* cells.



III.2. FOOD EXPOSURE

The loss kinetics of ^{63}Ni ingested with food in both the clam *G. tumidum* and the oyster *I. isognomon* were best fitted using a double exponential model (R^2 = 0.49 and 0.59, respectively) (Table 3 and Fig. 1-B). A substantial part of the ^{63}Ni activity (39 % for the clam and 83 % for the oyster) was rapidly lost via defecation ($T_{b½}$ < 2 d). Assimilation efficiency (AE) of ^{63}Ni was 61 % and 17 % in clams and oysters, respectively. These assimilated fractions were retained with $T_{b½}$ of 35 d in clams and a time not different from the infinite in oysters. During the depuration period, the highest proportion of ^{63}Ni (74 %) was associated with visceral mass + mantle in *I. isognomon* whereas in *G. tumidum* each organ contributed similarly to the global ^{63}Ni content (Fig. 2-B). In both clam and oyster, ^{63}Ni body distribution showed no major difference between the two sampling times (12 and 46 d).

Table 3. Assimilation efficiency (AE, %), loss rate constant (k_e, d^{-1}) and biological half life ($T_{b½}$, d) of ^{63}Ni in whole soft parts of the clam *Gafrarium tumidum* and the oyster *Isognomon isognomon,* after a 2-hr feeding on radiolabelled *Isochrysis galbana* cells.

ASE: asymptotic standard error; R^2: determination coefficient of the loss kinetics

Species	AE ± ASE	k_e ± ASE	$T_{b½}$ ± ASE	R^2
G. tumidum	61.2 ± 4.5 [b]	0.019 ± 0.04 [b]	35 ± 7	0.49
I. isognomon	17.1 ± 6.9 [a]	0.0001 ± 0.015 *	n.s.i.	0.59

Significance of the estimated parameters [a] $p < 0.05$, [b] $p < 0.0001$, * $p > 0.05$

n.s.i.: $T_{b½}$ not significantly different from infinite

IV. DISCUSSION

Bivalves are well known for their capacity to accumulate metals to quite high levels (e.g. Phillips 1976b). However, few studies have been devoted to Ni in marine bivalves (Friedrich & Fillice 1976; Hardy & Roesijadi 1982), in particular in tropical areas.

IV.1. SEAWATER PATHWAY

Ideally, a bioindicator should bioconcentrate contaminants proportionally to the dissolved metal concentration occurring in the environment. This implies that the concentration factor (CF) of a contaminant would remain constant over the concentration range to which the organism could be exposed in its environment (Phillips 1980, 1990).

Previous experimental investigations of Ni bioaccumulation in marine organisms generally considered exposure concentrations that were far above (several orders of magnitude) the natural ones (e.g. up to 80 mg Ni l^{-1}, Friedrich & Fillice 1976). The concentrations tested in the present study (up to 1400 ng added Ni l^{-1}) were selected in order to cover the whole concentration range that can be encountered in the coastal waters of the New Caledonian lagoon (Fernandez et al. 2002a). Results showed that the bioconcentration of Ni in the clam *G. tumidum* and in both oysters *M. regula* and *I. isognomon* was directly proportional to the Ni concentration in seawater virtually over the whole range of Ni concentrations tested. Similar observations were made for other elements in *I. isognomon* and *G. tumidum* such as Co, Cr, Mn also present in Ni ores (Hédouin et al. submitted-b). The capacity of Ni bioconcentration reported here in clams and oysters (CF_{14d} ranging from 70 to 284 ml g^{-1} dry wt) was quite less efficient than CFs observed in comparable experimental conditions for some metals such as Ag and Zn, which may reach values up to 200,000 (Hédouin et al. submitted-a). However, the observed CF are in the range of those reported in previous studies related to Ni in bivalves (4 ml g^{-1}dry wt in the clam *Prototheca staminea*, Hardy & Roesijadi 1982; from 10 to 607 ml g^{-1} dry wt in the mussel *Mytilus edulis,* Friedrich & Fillice 1976; from 156 to 336 ml g^{-1} wet wt in *Crassotrea virginica* and *M. edulis*, Zaroogian & Johnson 1984).

Results indicated that, in relative units, loss kinetics of Ni from the soft parts of *G. tumidum* and *M. regula* were also independent on the Ni concentrations to which the organisms were previously exposed. These observations are in agreement with those of Zaroogian & Johnson (1984) who reported that the loss rate of Ni was similar in oysters exposed to two different Ni treatments (5,000 and 10,000 ng Ni l^{-1}). In addition, our study showed that Ni was efficiently retained in both bivalve species, with biological half-lives not significantly different from the infinite. These values should of course be considered with caution due to the relatively short duration of the experiment (32 d). Nevertheless, they clearly indicate that both clams and oysters would be able to preserve information regarding their contamination history over a quite long timescale (several months).

The very close resemblance of the two oyster species *I. isognomon* and *M. regula*, both in their appearance and life mode (Yonge 1968) was also reflected in their bioaccumulation and depuration capacities for Co, Cr, Zn and, to a lesser extend, Cd (Hédouin et al. submitted-b). Similarly, the present work indicated that bioconcentration of dissolved Ni was quite similar in both species on a whole-body basis, although a slight decrease in CF was observed in *I. isognomon* at the highest Ni concentration tested. However, examination of CF values at the organ level indicated that gills of *I. isognomon* concentrate Ni much more efficiently (up to one order of magnitude) than those of *M. regula.* This suggests that although these two species are very closely related in many aspects at a macroscopic scale, mechanisms controlling Ni uptake could be quite different in *I. isognomon* and *M. regula*. Furthermore, previous studies have observed that gills are generally a major site of Ni intake in bivalves (e.g. Hardy & Roesijadi 1982; Wilson 1983a), indicating that such a difference between these two oysters would deserve further investigation.

IV.2. FOOD PATHWAY

Although it is now well documented that assimilation of metals ingested with food plays an important role in their bioaccumulation in marine organisms (e.g. Wang & Fisher 1999b), very few studies have been devoted to trophic transfer of Ni. Kumblad et al. (2005) have shown that assimilation efficiency (AE) of sediment-associated ^{63}Ni (II) ranged from 43 to 49 % in the clam *Macoma balthica,* the amphipod *Monoporeia affinis* and the priapulid *Halicryptus spinulosus.* In the present study, estimated AE of ^{63}Ni was much higher in the clam *G. tumidum* (61 %) than in the oyster *I. isognomon* (17 %). Such a low assimilation in *I. isognomon* compared to *G. tumidum* as well as to the species studied by Kumblad et al. (2005) could be related to the differences in feeding physiology (e.g. digestion efficiency) of these different organisms (Lee II 1991; Mayer et al. 2001). However, in a recent study on other metals, AEs reaching values up to 77 % were found for Ag in *I. isognomon* (Hédouin et al. to be submitted-b), suggesting that low AE would not be a physiological characteristic of this species. Rather, the low assimilation of Ni in *I. isognomon* could be related on how the oyster behaves towards Ni and/or on how it is able to deal with the way Ni is bound to algal cells.

To the best of our knowledge, the estimated AEs for Ni are the very first ones published for the considered species. However, it has to be kept in mind that these Ni AEs were obtained in controlled feeding conditions (mono-specific culture of *I. galbana* at 10^4 cell ml^{-1}). These

conditions are quite different from those found in the field which are much more complex and variable and which could result in actual AEs somewhat departing from those estimated here. It is indeed documented that AE may be influenced by the food sources considered (Wang & Fisher 1999a; Chong & Wang 2000a). This has also been recently observed for Co, Mn and Zn in *G. tumidum* and *I. isognomon*: AEs estimated for these 3 metals with 3 different strains of phytoplankton were found to vary over a factor 4 (Hédouin et al. submitted-c). Hence, Ni AEs estimated in the present study have to be considered as preliminary and other feeding conditions should be investigated in case they would be used for generalisation to environmental conditions.

IV.3. CONCLUSIONS

Within a range of Ni dissolved concentrations that cover the natural range occurring in New Caledonia seawaters, (1) Ni bioconcentration was directly proportional to the Ni concentration in seawater for *G. tumidum, M. regula* and, to a lesser extend, *I. isognomon*, and (2) the retention efficiency of Ni in clams and oysters was independent on the total Ni concentration previously accumulated by the organisms. In addition, clams and oysters were shown to assimilate efficiently Ni ingested with their food (in particular in clams) and retained it quite efficiently (in particular in oysters). These characteristics suggest that these New Caledonian bivalves could be used to detect Ni contamination in their surrounding environment and to preserve this information over long periods of time. In addition, the clams and oysters displayed different bioaccumulation behaviour for Ni, especially when exposed via the food. Both groups would thus be worth to be further characterized with regard to their values as bioindicators for Ni contamination. Indeed, both groups could provide complementary ecotoxicological information as they interact differently with their environment.

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CHAPITRE 5

Influence de la concentration en éléments dissouts sur leur bioconcentration dans des organismes marins du lagon de Nouvelle-Calédonie : Validation de leur utilisation en tant qu'espèces bioindicatrices

L'huître *Isognomon isognomon* et le clam *Gafrarium tumidum* ont été proposés comme espèces bioindicatrices potentielles de la contamination métallique dans le lagon Sud Ouest de Nouvelle-Calédonie. L'influence des concentrations dissoutes en As, Cd, Co, Cr, Mn et Zn sur les cinétiques d'accumulation et d'élimination, les distributions corporelles et subcellulaires des éléments a été étudiée chez les deux espèces. Les résultats indiquent que les bivalves accumulent les éléments proportionnellement à leur concentration dissoute dans l'eau ambiante pour le Cd, Cr et Mn sur toute la gamme de concentration étudiées (trois ordres de grandeur), et jusqu'à la deuxième concentration la plus élevée pour le Co et le Zn. Tous les éléments sont efficacement retenus dans les tissus des bivalves (les $T_{b½}$ estimés varient de 16 j à un temps non différent de l'infini), ce qui suggère que les deux espèces devraient être capables de préserver l'information liée aux événements contaminants sur une longue période de temps. Compte tenu des gammes de concentrations considérées, *G. tumidum* et *I. isognomon* peuvent constituer d'excellentes espèces bioindicatrices pour la surveillance de la contamination métallique dans le lagon de Nouvelle-Calédonie.

Influence of dissolved element concentration on the bioconcentration of marine organisms from New Caledonia lagoon: Validation of their use as bioindicator species

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ABSTRACT. The oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* have been proposed as potential bioindicator species of metal contamination in the New Caledonia lagoon. The influence of dissolved concentrations of As, Cd, Co, Cr, Mn and Zn, and on uptake and loss kinetics, body and subcellular distribution of the elements has been investigated in both species. Results indicate that bivalves took up elements proportionally to the ambient dissolved concentration for Cd, Cr and Mn over the whole range of concentration tested (three orders of magnitude), and up to the second highest concentration tested for Co and Zn. All elements were efficiently retained in bivalve tissues (estimated $T_{b½}$ ranging from 16 d to ∞), suggesting that both species should be able to preserve information of contamination events over a long period of time. Considering the specific range of concentration determined here, *G. tumidum* and *I. isognomon* would therefore constitute excellent bioindicator species to monitor metal contamination in the New Caledonia lagoon.

Keywords: Bivalve, Tropical, Metal, Bioconcentration, Bioindicator, Radiotracer techniques

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I. INTRODUCTION

The coral barrier of New Caledonia encloses the second largest lagoon of the world after the Australian Great Barrier Reef (Labrosse et al. 2000). Beside a rich biodiversity and a high endemism (approximately 5 % of the 15,000 recorded species; Richer de Forges et al. 2000; Bouchet et al. 2002), the New Caledonia lagoon is also subjected to high contamination pressure. Indeed, about 25 % of the world nickel stocks are present in New Caledonia (Dalvi et al. 2004). Ores have been locally exploited since the end of the nineteenth century, mainly to extract Ni. Nowadays, mining activities constitute the most important economical resources of the Territory. Recent development of more efficient extraction processes based on acidic extraction (viz. lixiviation) is now making possible extraction from ores with lower Ni contents than those currently exploited (Mihaylov et al. 2000; Goro-Nickel 2001; Dalvi et al. 2004). The acidic solubilization of metals is obviously not be restricted to Ni which is exploited but also concerns all other ore-contained by-product metals. Therefore there is a non-negligible risk that application o-f lixiviation process will eventually result in increasing discharges of dissolved by-product metals into the environment (Goro-Nickel 2001; Baroudi et al. 2003). In particular, Co, Cr, and Mn (viz. the most abundant by-products) in New Caledonia mines should be considered with special caution (Bird et al. 1984).

Even though increase of metal contamination in coastal waters is a primary threatening consequence of mining activities, so far little attention has been given to possible impact on the local marine ecosystems. Furthermore, studies reporting metal concentrations in tropical organisms from New Caledonian are scarce and generally preliminary (Monniot et al. 1994; Bustamante et al. 2000; Breau 2003).

Among the common approaches used to study environmental contamination, the use of bioindicator species has been proven to be valuable and informative. This approach has received much scientific interest in the temperate zone with the implementation of e.g., Mussel Watch programmes (Goldberg et al. 1978). Yet, only few studies have been dedicated to biomonitoring development and implementation in tropical zones. Although some studies have proposed bivalves such as *Saccostrea* spp., *Crassostrea* spp. or *Perna* spp. as potential tropical bioindicators (e.g. Phillips 1985; Rainbow & Phillips 1993), none of these species are present in sufficient abundance in the New Caledonia lagoon to be considered as interesting bioindicators. In this context, the infaunal clam *Gafrarium tumidum* and the oyster *Isognomon*

isognomon have been proposed as a potential target species. Indeed, they respond to most criteria that should be met by a bioindicator species (Breau 2003; Hédouin et al. submitted-b). Among the pre-requisites of a bioindicator organism (see e.g. Moore 1966; Phillips 1980, 1990), the selected species should display a simple relationship between contaminants accumulated in its tissues and the concentration of these contaminants in the environment. Although this last recommendation is of major importance, the effect of ambient metal concentration on the bioconcentration capacities of organisms has rarely been assessed (Borchardt 1983; Talbot 1985; Bjerregaard 1988; Warnau et al. 1997). In the New Caledonian lagoon, this is of particular concern, since very wide ranges of metal concentrations have been reported in coastal waters (two orders of magnitude; Fernandez et al. 2002a).

Therefore, the purpose of this study was to investigate the effect of dissolved element concentration on the bioconcentration of the infaunal clam *G. tumidum* and the oyster *I. isognomon* exposed to realistic concentrations of Co, Zn, Mn, Cd, Cr, and As in seawater. Although metal bioaccumulation in marine bivalves is well known to occur simultaneously from several pathways (seawater, suspended particles and food), the present study concentrates on the direct uptake from seawater. Indeed, recent reports indicate that contaminants in the effluents from lixiviation processes are mainly present under dissolved forms (Goro-Nickel 2003; Stauber et al. 2003). Particular attention was given to the determination of the influence of ambient concentrations on bioconcentration, body distribution and subcellular distribution of the considered elements.

II. MATERIALS AND METHODS

II.1. COLLECTION AND ACCLIMATION

The clams *Gafrarium tumidum* were collected by hand-picking in October 2003, in Dumbéa Bay, Nouméa, New Caledonia (n = 300; whole-body wet wt from 17.7 to 28.0 g). The oysters *Isognomon isognomon* were collected by SCUBA diving in October 2003, in Maa Bay, Nouméa, New Caledonia (n = 300; whole-body wet wt from 22.1 to 42.2 g). Body size is well known to affect bioaccumulation of metals in marine organisms (e.g. Boyden 1974). Therefore, according to preliminary studies (Metian 2003; Hédouin et al. in press), only organisms with shell width longer than 35 mm (*G. tumidum*) and shell length longer than 70 mm (*I. isognomon*) were collected. Oysters and clams were shipped to IAEA-MEL premises in Monaco, where they were acclimated to laboratory conditions (open circuit aquarium;

water renewal rate: 30 % hr^{-1}; salinity: 36 p.s.u.; temperature T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) simulating the conditions prevailing in New Caledonian lagoon. During the 2 months of acclimation, bivalves were fed phytoplankton (*Isochrysis galbana);* recorded mortality was lower than 5 %.

II.2. STABLE ELEMENTS, RADIOTRACERS AND RADIOANALYSES

Investigated elements (Cr, Mn, Co, Zn, As and Cd) were introduced into the experimental microcosms both as stable elements and radiotracers (^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd) to detect elements with high sensitivity. Stable elements were introduced as HNO_3 salts (synthesis quality) purchased from Merck, France. Radiotracers of high specific activity were purchased from Amersham, UK (^{51}Cr as Na_2CrO_4, $T_{½}$ = 27.7 d; ^{57}Co as $CoCl_2$, $T_{½}$ = 271.8 d), Isotope Product Lab., USA (^{109}Cd as $CdCl_2$, $T_{½}$ = 426.6 d; ^{65}Zn as $ZnCl_2$; $T_{½}$ = 243.9 d; ^{54}Mn as $MnCl_2$, $T_{½}$ = 312.2 d), Hyenryk Niewodniczanski Institute of Nuclear Physics of the Polish Academy of Sciences (^{73}As as $HAsO_3$, $T_{½}$ = 80.3 d). Radioactivity was measured using a high-resolution γ-spectrometer system (4 Germanium-N or P type-detectors: EGNC 33-195-R, Intertechnique) connected to a multichannel analyser (Intergamma, Intertechnique). The radioactivity of samples was determined by comparison with standards of known activities and of appropriate geometry. Measurements were corrected for counting efficiency, background and radioactive decay. Counting time was adapted to obtain counting rates with propagated errors less than 5 %.

II.3. EXPERIMENTAL PROCEDURE

For each element tested, 5 groups of 9 clams (shell width from 35 to 44 mm), and 5 groups of 9 oysters (shell length from 74 to 112 mm) were dispatched in 5 aquaria containing 50 l of natural seawater (salinity: 36 p.s.u.; T° = 25 ± 0.5°C; pH = 8.0 ± 0.1). Bivalves were exposed for 14 d to five increasing added concentrations of a given element, up to 1250 ng Cr l^{-1}, 998 ng Co l^{-1}, 1250 ng Mn l^{-1}, 1750 ng Zn l^{-1}, 10000 ng As l^{-1} and 250 ng Cd l^{-1} (Table 1). These added concentrations were realized using increasing amount of the stable element and a fixed activity of the corresponding radiotracer. The concentrations were selected in order to cover the concentrations ranges representative of those actually encountered in the New Caledonia lagoon (Fernandez et al. 2002a). Spikes of radiotracer were: ^{51}Cr (1.5 kBq l^{-1}), ^{54}Mn (0.5 kBq l^{-1}), ^{57}Co (0.5 kBq l^{-1}), ^{65}Zn (0.5 kBq l^{-1}), ^{73}As (0.5 kBq l^{-1}) and ^{109}Cd (1 kBq l^{-1}). In terms of

stable element additions, these activities correspond to Cr ($1.39\ 10^{-10}$ g l^{-1}), Mn ($3.55\ 10^{-10}$ g l^{-1}), Co ($2.75\ 10^{-11}$ g l^{-1}), Zn ($5.99\ 10^{-9}$ g l^{-1}), As ($2.30\ 10^{-30}$ g l^{-1}) and Cd ($4.84\ 10^{-11}$ g l^{-1}).

Table 1. Concentrations of the six stable elements investigated and their corresponding radiotracers added in seawater for preparing the increasing exposure concentrations (C0-C4).

Radiotracers		Stable metal concentrations (ng l^{-1})				
	kBq l^{-1}	C0	C1	C2	C3	C4
As	0.5	0	2000	5000	10000	
Cd	1.0	0	2	10	50	250
Co	0.5	0	8	23	248	998
Cr	1.5	0	10	50	250	1250
Mn	0.5	0	10	50	250	1250
Zn	0.5	0	70	250	700	1750

No change in pH was detectable after radiotracer and element additions. Seawater and spikes were renewed daily for 5 d, then every second day in order to keep exposure activities as constant as possible. Concentrations of the element in seawater were checked daily, before and after each seawater renewal using the radiotracer activity. This allowed calculation of the actual element concentrations based on the ratio between nominal concentration of the element and the nominal activity of the corresponding tracer. The decrease in seawater radioactivity between two successive seawater renewals (mean decrease ± SD, n = 28) was 10 ± 6 % for ^{51}Cr, 23 ± 11 % for ^{54}Mn, 25 ± 11 % for ^{57}Co, 21 ± 12 % for ^{65}Zn, 11 ± 10 % for ^{73}As and 13 ± 11 % for ^{109}Cd.

During the experiment duration, clams were allowed to feed briefly (30 min) on phytoplankton *Isochrysis galbana* in clean seawater every second day, before a seawater and spike renewal in order to minimize ingestion of metal via the food. At different time intervals, organisms were collected and whole-body γ-counted (alive) in order to determine radiotracer uptake kinetics in each individual, and replaced in their aquarium. At the end of the period of exposure (14 d), 3 individuals of each species were sacrificed and soft parts were separated from shells. Clam soft parts were then dissected in 6 body compartments (mantle, muscle, foot, gills, hepatopancreas and remaining soft tissues) and oysters in 4 body compartments

(visceral mass, gills, muscle and remaining soft tissues). Body compartments were then weighed (wet weight) and radioanalysed to measure their respective radiotracer activities and to assess the element body distribution. In the digestive organs (digestive gland and visceral mass for clams and oysters, respectively) and gills of both bivalves, the subcellular distribution of all radiotracers was investigated between soluble and insoluble fractions according to the method described by Bustamante & Miramand (2005). Briefly, organs were homogenized individually with a mortar and pestle on ice with 10 volumes of 0.02 M Tris–HCl buffer, 0.25 M sucrose, 1 mM phenylmethylsulfonylfluoride (PMSF, as protease inhibitor), at pH 8.6. The homogenates were centrifuged at 44700 × g for 2 h 30 at 5°C in a Sorvall RC28S ultracentrifuge to separate particle-free supernatant (cytosol) from the pellet. Homogenate aliquots, cytosols, and pellets were then radioanalysed.

The remaining bivalves were then placed in non-contaminated conditions (50-l aquaria with flowing natural seawater, flux: 50 % hr^{-1}, salinity: 36 p.s.u.; T° = 25 ± 0.5°C; light/dark cycle: 12 hrs/12 hrs) for 21 d to follow the loss of radiotracers from whole-body organisms. At the end of depuration period (21 d), all individuals were dissected to determine the element distribution among body compartments as described above.

II.4. DATA TREATMENT

Uptake of the five investigated radiotracers was expressed in terms of concentration factor (CF, ratio between activity of the radiotracer in the whole organism or in a body compartment -Bq g^{-1} wet weight- and time-integrated activity of radiotracer in seawater -Bq g^{-1}-). Radiotracer uptake kinetics were described using a simple linear regression model (eq.1) or, if the observed kinetics tended to reach a steady-state equilibrium, using a saturation exponential kinetic model (eq.2):

$CF_t = k_u\, t$ (eq.1)

$CF_t = CF_{ss}\,(1-e^{-k_e\, t})$ (eq.2)

where CF_t and CF_{ss} are the concentration factors at time t (d) and at steady state, respectively; k_u and k_e are the uptake and loss rate constants (d^{-1}), respectively (Whicker & Schultz 1982). Linearity of the uptake kinetics was tested by a linearity test for regression with replication (Zar 1996). Model constants (CF_{ss}, k_u and k_e) were estimated by iterative adjustment of the model using the nonlinear curve-fitting routines in the Statistica software 5.2.1.

Loss kinetics of radiotracers were expressed as the percentage of remaining radioactivity (radioactivity at time t divided by the initial radioactivity measured in the organism at the beginning of the depuration period). The loss kinetics of radiotracers were best fitted by either a single-component (eq.3) or a double-component exponential equation (eq.4):

$A_t = A_0 e^{-k_e t}$ (eq.3)

$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t}$ (eq.4)

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components, respectively. The short-lived component represents the loss kinetics of the radiotracer fraction that is weakly associated with organisms and rapidly eliminated, whereas the long-lived component describes the loss kinetics of the radiotracer fraction that is tightly bound to the organism. For each exponential component (s and l), a biological half-life can be calculated ($T_{b½s}$ and $T_{b½l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b½} = \ln 2/k_e$. The biological half-life represents the time necessary for the organism to eliminate 50 % of the amount of radiotracer accumulated.

Differences among uptake and loss kinetics of each radiotracer along the concentration range considered were tested using the Linear Mixed Models (LMM) procedure implemented in SPSS v12.0 software. This procedure expands the general linear model so that the error terms and random effects are permitted to exhibit correlated and non-constant variability. The LMM, therefore, provides the flexibility to model not only the mean of a response variable, but its covariance structure as well. Time was specified as the repeated effect and the total dissolved concentrations (stable + radioactive) of Cr, Co, Mn, Zn, As and Cd as the fixed effect.

Estimated kinetic parameters (k_u, k_e, CF_{ss}, A_{0l}, A_{0s}, k_{es}, k_{el}) were plotted against the concentration of total element (stable + radioactive) in seawater and were fitted using simple linear regression (Statistica 5.2.1 software).

The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

III.1. CLAM *G. TUMIDUM*

Whole-body uptake kinetics of all radiotracers in clams displayed saturation kinetics for all concentrations tested (R^2: 0.82-0.95 for ^{51}Cr, ^{54}Mn, ^{65}Zn; 0.68-0.91 for ^{57}Co and ^{109}Cd; and 0.48-0.66 for ^{73}As), except for ^{54}Mn which was accumulated according to linear uptake kinetics (R^2: 0.90-0.94) (Fig. 1, Table 2). Comparisons among the rates (k_u) at which radiotracers were concentrated in the whole-body organisms indicated that the six radiotracers ranked according to the following decreasing order: $^{54}Mn > ^{65}Zn > ^{57}Co > ^{73}As > ^{109}Cd > ^{51}Cr$. The estimated steady-state concentration factors (CF_{ss}) indicated that the radiotracers were concentrated to different extent by the clam *G. tumidum*, according to the following order: $^{57}Co, ^{65}Zn > ^{51}Cr, ^{109}Cd > ^{73}As$ (Table 2).

Linear Mixed Model (LMM) analyses indicated that uptake kinetics of ^{51}Cr, ^{54}Mn and ^{109}Cd did not significantly differ ($p_{SPSS} > 0.05$) on the range of concentrations tested. In contrast, exposure concentration in seawater affected significantly the uptake kinetics of ^{57}Co, ^{65}Zn and ^{73}As in clams ($p_{SPSS} < 0.0001$, except for ^{73}As $p_{SPSS} = 0.03$). For Co concentration higher than 23 ng l^{-1}(C_2) loss rate constant (k_e) significantly increased ($p < 0.001$), whereas CFss decreased ($p < 0.05$). Effect of the exposure concentration in seawater on uptake kinetics of As was observed for concentration exceeding 5000 ng l^{-1} (C_2): loss rate constant (k_e) significantly increased ($p < 0.005$). For Zn, decrease in k_e value by one order of magnitude was observed between the control concentration (0 ng added Zn l^{-1}) and the other concentrations tested.

The distribution of radiotracers among the clam body compartments was determined at the end of exposure period in the five conditions tested (Fig. 2). Body distribution was slightly affected by exposure concentration for all elements, except Co. Indeed, when the dissolved Co concentration was higher than 23 ng l^{-1} (C_2), whereas the amount of Co in digestive gland decreases, the amount of Co in mantle and gills increases.

Table 2. Estimated uptake rate constant (k_u, d^{-1}) and concentration factor at steady state (CF_{ss}) of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in the clam *Gafrarium tumidum* exposed to five increasing dissolved concentration for 14 d.

L: Linear model: $CF_t = k_u\, t$

E: Exponential model: $CF_t = CF_{ss}\,(1\text{-}e^{-k_e\, t})$

ASE: asymptotic standard error; R^2: determination coefficient of the uptake kinetics

Isotopes	Model	Concentration	k_u ± ASE	CF_{ss} ± ASE	R^2
^{51}Cr	E	C0	0.86 ± 0.05 ^{d}	8.3 ± 0.6 ^{d}	0.94
	E	C1	0.79 ± 0.05 ^{d}	34.2 ± 14.1 ^{a}	0.95
	E	C2	0.63 ± 0.04 ^{d}	13.1 ± 2.6 ^{d}	0.93
	E	C3	0.78 ± 0.05 ^{d}	12.0 ± 1.6 ^{d}	0.93
	E	C4	1.19 ± 0.12 ^{d}	9.9 ± 1.0 ^{d}	0.85
^{54}Mn	L	C0	9.81 ± 0.18 ^{d}		0.93
	L	C1	10.11 ± 0.17 ^{d}		0.94
	L	C2	11.08 ± 0.19 ^{d}		0.94
	L	C3	9.26 ± 0.20 ^{d}		0.90
	L	C4	11.48 ± 0.20 ^{d}		0.94
^{57}Co	E	C0	6.29 ± 0.42 ^{d}	121 ± 23 ^{d}	0.90
	E	C1	6.10 ± 0.61 ^{d}	129 ± 40 ^{b}	0.86
	E	C2	5.72 ± 0.48 ^{d}	141 ± 44 ^{b}	0.91
	E	C3	5.64 ± 0.58 ^{d}	54.8 ± 7.1 ^{d}	0.86
	E	C4	5.34 ± 0.20 ^{d}	26.1 ± 1.8 ^{d}	0.75
^{65}Zn	E	C0	7.95 ± 0.61 ^{d}	294 ± 129 ^{a}	0.92
	E	C1	9.6 ± 0.6 ^{d}	96.7 ± 9.1 ^{d}	0.92
	E	C2	9.1 ± 1.0 ^{d}	106 ± 18 ^{d}	0.82
	E	C3	7.44 ± 0.52 ^{d}	77.4 ± 7.4 ^{d}	0.91
	E	C4	6.12 ± 0.06 ^{d}	105 ± 24 ^{d}	0.87
^{73}As	E	C0	2.16 ± 0.29 ^{d}	7.2 ± 0.5 ^{d}	0.66
	E	C1	2.70 ± 0.36 ^{d}	7.8 ± 0.4 ^{d}	0.65
	E	C2	2.21 ± 0.37 ^{d}	5.0 ± 0.3 ^{d}	0.53
	E	C3	2.97 ± 0.59 ^{d}	4.6 ± 0.2 ^{d}	0.48
^{109}Cd	E	C0	1.44 ± 0.16 ^{d}	10.8 ± 1.4 ^{d}	0.80
	E	C1	1.30 ± 0.11 ^{d}	8.3 ± 0.6 ^{d}	0.87
	E	C2	1.33 ± 0.12 ^{d}	7.4 ± 0.5 ^{d}	0.85
	E	C3	1.31 ± 0.19 ^{d}	7.8 ± 1.0 ^{d}	0.68
	E	C4	1.46 ± 0.19 ^{d}	7.8 ± 0.6 ^{d}	0.73

Significance of the estimated parameters: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$, * $p > 0.05$

Figure 1. ***Gafrarium tumidum*****. Influence of stable Cr, Mn, Co, Zn, As and Cd concentrations in seawater on whole-body uptake of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in clams (mean concentration factor, CF, ± SD, n = 9).**

Parameters and statistics of the uptake kinetics are given in Table 2.

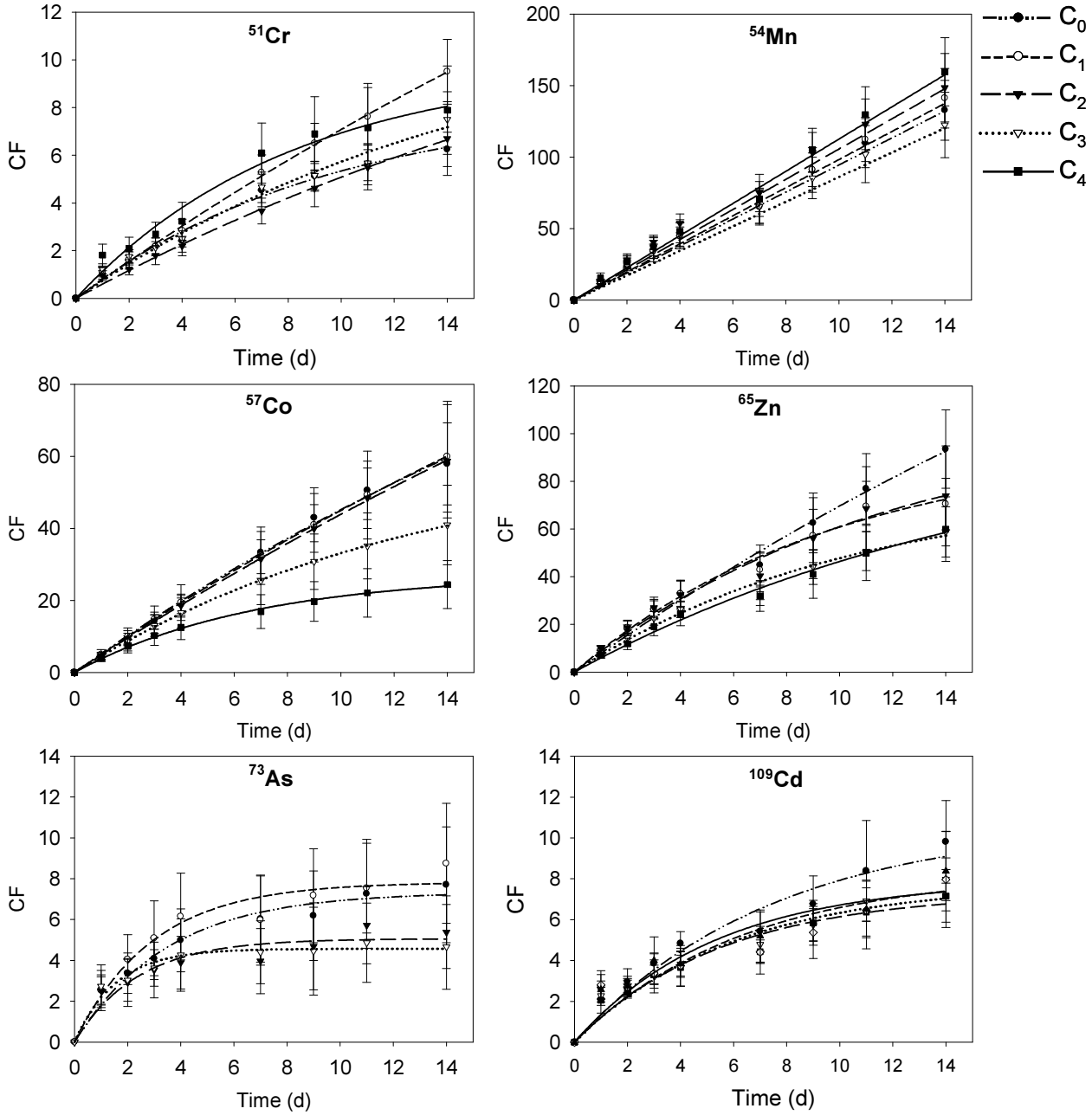


Figure 2. ***Gafrarium tumidum*****. Radiotracer distribution (mean % ± SD, n = 3) among the different body compartments of the clams exposed for 14 d to 5 increasing concentrations (C0-C4) of Cr, Mn, Co, Zn, As and Cd in seawater.**

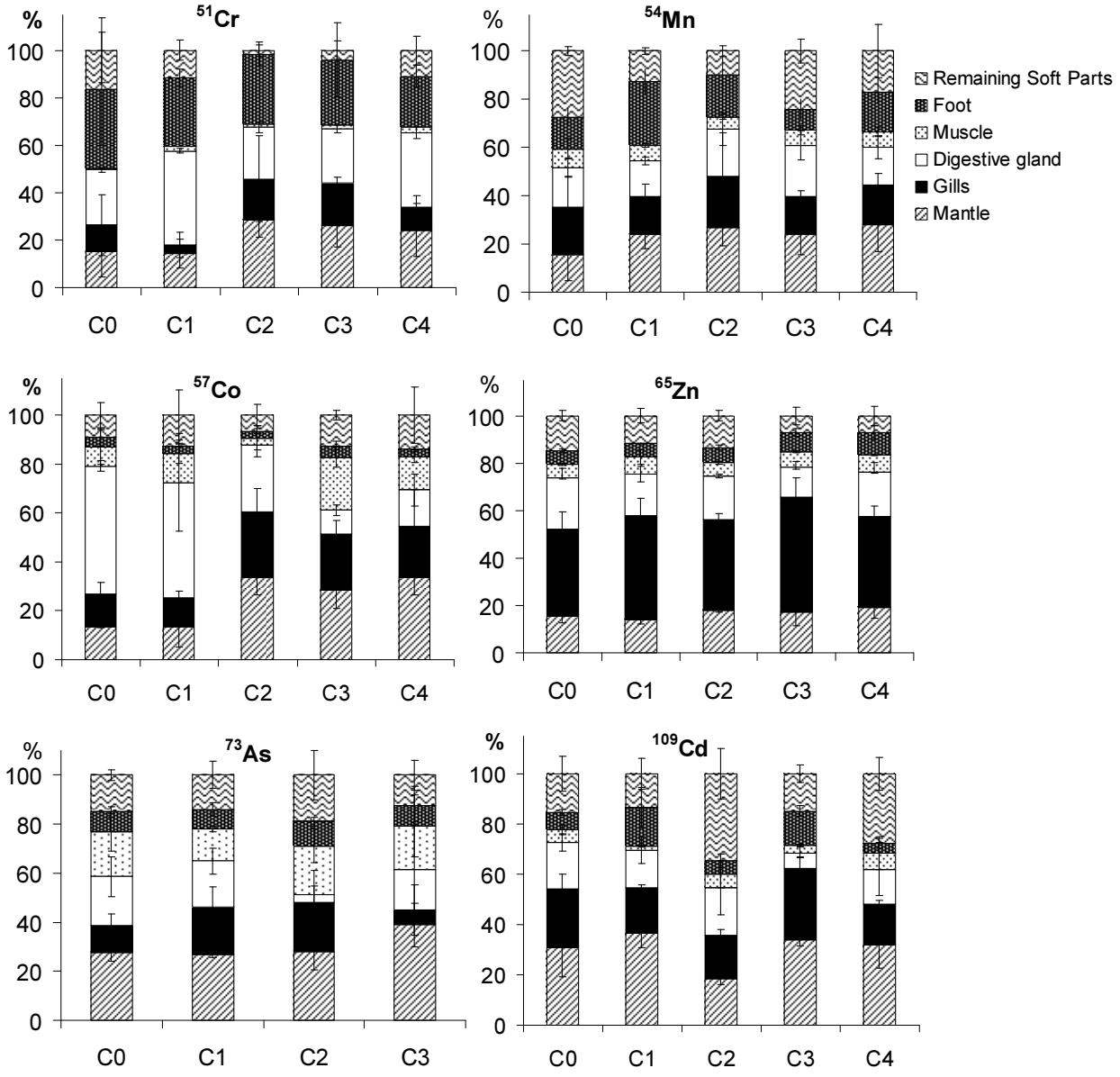
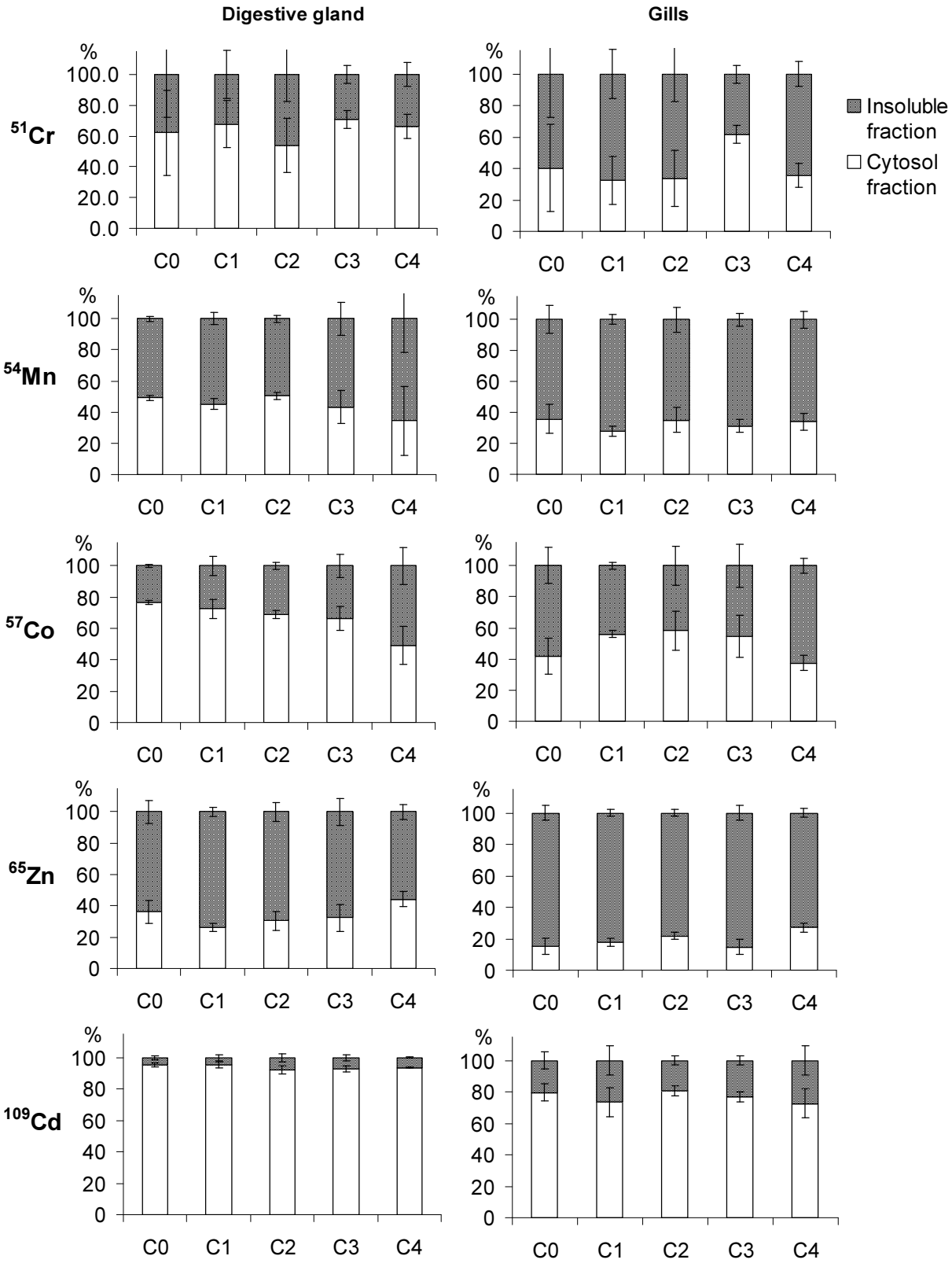


Figure 3. ***Gafrarium tumidum*****. Subcellular distribution (mean % ± SD, n = 3) of radiotracers in the digestive gland and gills of the clams exposed for 14 d to 5 increasing concentrations (C0-C4) of Cr, Mn, Co, Zn and Cd in seawater.**



No major difference was observed in the subcellular distribution of all radiotracers investigated in digestive organs and gills of clams exposed to the five increasing concentrations. The examination of these distributions between soluble and insoluble fraction (Fig. 3) indicated that in both digestive gland and gills, ^{109}Cd was mainly associated with the cytosolic fraction, ranging from 73 to 95 %, whereas ^{65}Zn was mostly bound to insoluble compounds (56 to 85 %). The major part of ^{51}Cr and ^{54}Mn was associated with insoluble fraction in the gills. In the digestive gland, ^{51}Cr and ^{57}Co were mainly (49 to 77 %) associated with insoluble compounds.

After the 14-d exposure period, bivalves were maintained for 21 d in clean flowing seawater to follow loss of radiotracers. Loss kinetics of ^{51}Cr, ^{54}Mn and ^{57}Co were best fitted by a single-component exponential model for all concentrations tested (Fig. 4, Table 3).

Loss kinetics for the three other radiotracers (^{65}Zn, ^{73}As and ^{109}Cd) were best described by a two-component exponential model. No significant difference ($p_{SPSS} > 0.05$) was observed among the loss kinetics determined along the concentration range examined. The long-lived loss components of ^{65}Zn and ^{109}Cd represented a large proportion of the radioactivity (accounting for 78 to 87 % and 69 to 79 %, for Zn and Cd, respectively) and was characterized by a $T_{b½}$ ranging from 45 to 56 d for ^{65}Zn, and by an infinite $T_{b½}$ for ^{109}Cd. Similar proportion of radioactivity was attributed to the long and short-lived loss components of ^{73}As; the long-lived loss component indicated that ^{73}As was retained with $T_{b½}$ ranging from 16 to 54 d. The estimated $T_{b½}$ indicated that radiotracers were retained with different efficiency in the clams *G. tumidum*, ranking as follows by order of decreasing retention time: Cd > Cr = Zn > Mn > Co > As.

At the end of the depuration period, the distribution of radiotracers among the clam body compartments (Fig. 5) was generally similar for the different concentrations tested, except for ^{57}Co. For this latter element, the fraction of ^{57}Co significantly increased in gills and decreased in foot with increasing Co concentration in seawater. This suggests that loss rate of Co would increase from foot when organisms are exposed to higher concentrations. The distribution of radiotracers among the clam body compartments were also similar to those found at the end of exposure period for ^{54}Mn, ^{65}Zn and ^{109}Cd, suggesting that the loss of these three elements proceeds at similar rates in the different body compartments. For ^{51}Cr, an important proportion of the metal was associated with muscles at the end of the depuration period (Fig. 5).

Table 3. Parameters of the loss kinetics of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in the clam *Gafrarium tumidum* previously exposed for 14 d to radiotracers via seawater.

S: Single-component loss exponential model: $A_t = A_0 e^{-k_e t}$;

T: Two-component loss exponential model: $A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t}$

A_0 (%): Absorption efficiency, k_e (d^{-1}): loss rate constant and $T_{b½}$ (d): biological half life (when loss kinetics followed a T model, only A_{0l}, k_{el} and $T_{b½l}$ are indicated)

ASE: asymptotic standard error; R^2: determination coefficient of the loss kinetics

Isotopes	Model	Concentration	A_0 ± ASE	k_e ± ASE	$T_{b½}$ ± ASE	R^2
^{51}Cr	S	C0	96.7 ± 1.2 ^{d}	0.017 ± 0.001 ^{d}	41.9 ± 3.5	0.72
	S	C1	96.9 ± 1.1 ^{d}	0.018 ± 0.001 ^{d}	38.6 ± 2.7	0.83
	S	C2	96.3 ± 1.5 ^{d}	0.014 ± 0.002 ^{d}	50.3 ± 5.7	0.59
	S	C3	96.5 ± 1.2 ^{d}	0.017 ± 0.001 ^{d}	40.3 ± 3.1	0.76
	S	C4	95.4 ± 1.2 ^{d}	0.012 ± 0.001 ^{d}	56.0 ± 5.6	0.64
^{54}Mn	S	C0	101.1 ± 0.7 ^{d}	0.022 ± 0.001 ^{d}	32.1 ± 1.0	0.94
	S	C1	99.9 ± 0.5 ^{d}	0.020 ± 0.001 ^{d}	35.0 ± 0.9	0.96
	S	C2	101.3 ± 0.4 ^{d}	0.019 ± 0.005 ^{d}	37.0 ± 0.9	0.96
	S	C3	101.7 ± 0.8 ^{d}	0.020 ± 0.001 ^{d}	33.9 ± 1.3	0.91
	S	C4	100.6 ± 0.7 ^{d}	0.019 ± 0.001 ^{d}	35.6 ± 1.4	0.91
^{57}Co	S	C0	101.1 ± 0.6 ^{d}	0.027 ± 0.001 ^{d}	25.2 ± 0.6	0.96
	S	C1	102.0 ± 0.6 ^{d}	0.021 ± 0.001 ^{d}	33.8 ± 1.0	0.95
	S	C2	101.2 ± 0.8 ^{d}	0.023 ± 0.001 ^{d}	30.1 ± 1.1	0.93
	S	C3	100.1 ± 0.5 ^{d}	0.026 ± 0.001 ^{d}	26.9 ± 0.7	0.97
	S	C4	98.1 ± 1.1 ^{d}	0.021 ± 0.001 ^{d}	32.4 ± 1.9	0.83
^{65}Zn	T	C0	85.5 ± 2.1 ^{d}	0.015 ± 0.002 ^{d}	47.7 ± 5.9	0.91
	T	C1	86.7 ± 5.6 ^{d}	0.014 ± 0.004 ^{b}	48.2 ± 14.6	0.77
	T	C2	87.3 ± 2.7 ^{d}	0.015 ± 0.002 ^{d}	45.1 ± 6.7	0.83
	T	C3	81.7 ± 5.1 ^{d}	0.015 ± 0.004 ^{c}	45.0 ± 12.7	0.79
	T	C4	78.3 ± 4.9 ^{d}	0.012 ± 0.004 ^{b}	55.9 ± 19.3	0.85
^{73}As	T	C0	50.6 ± 6.5 ^{d}	0.037 ± 0.009 ^{c}	18.9 ± 4.7	0.95
	T	C1	53.1 ± 6.5 ^{d}	0.043 ± 0.009 ^{d}	16.2 ± 3.4	0.92
	T	C2	36.3 ± 12.6 ^{b}	0.013 ± 0.022 *	54.2 ± 92	0.93
	T	C3	54.4 ± 10.4 ^{d}	0.036 ± 0.012 ^{b}	19.3 ± 6.7	0.85
^{109}Cd	T	C0	78.5 ± 3.9 ^{d}	0.004 ± 0.003 *	177 ± 152	0.71
	T	C1	69.8 ± 4.2 ^{d}	0.002 ± 0.004 *	347 ± 693	0.60
	T	C2	76.5 ± 8.0 ^{d}	0.001 ± 0.007 *	1226 ± 14381	0.45
	T	C3	68.8 ± 63.9 *	0.003 ± 0.052 *	231 ± 4005	0.70
	T	C4	76.6 ± 4.9 ^{d}	0.005 ± 0.004 *	139 ± 122	0.62

Significance of the estimated parameters: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$, * $p > 0.05$

Figure 4. ***Gafrarium tumidum*****. Influence of stable Cr, Mn, Co, Zn, As and Cd concentrations in seawater on whole-body loss of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in clams (mean % remaining activities, A (%), ± SD, n = 6).**

Parameters and statistics of the loss kinetics are given in Table 3.

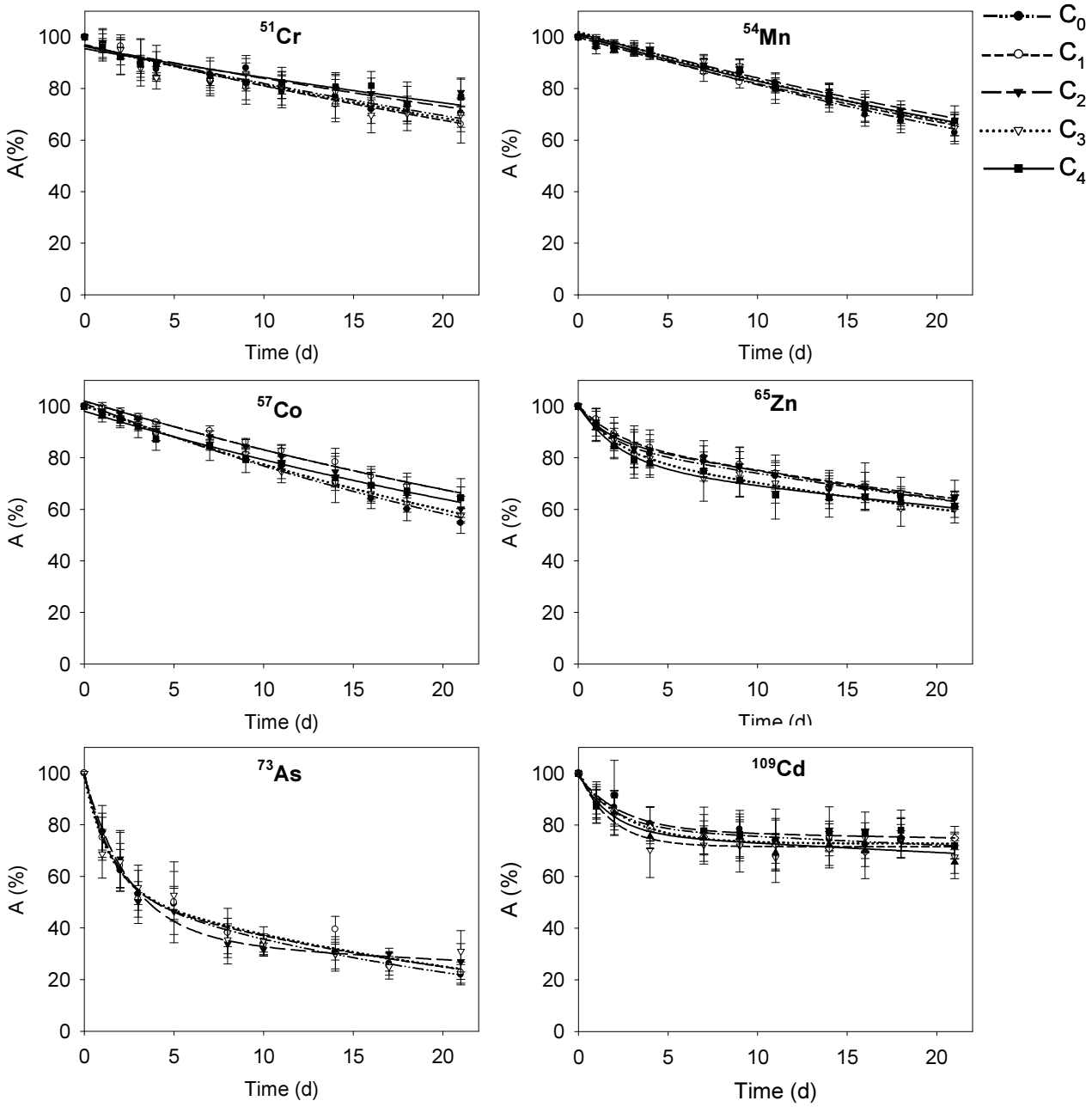
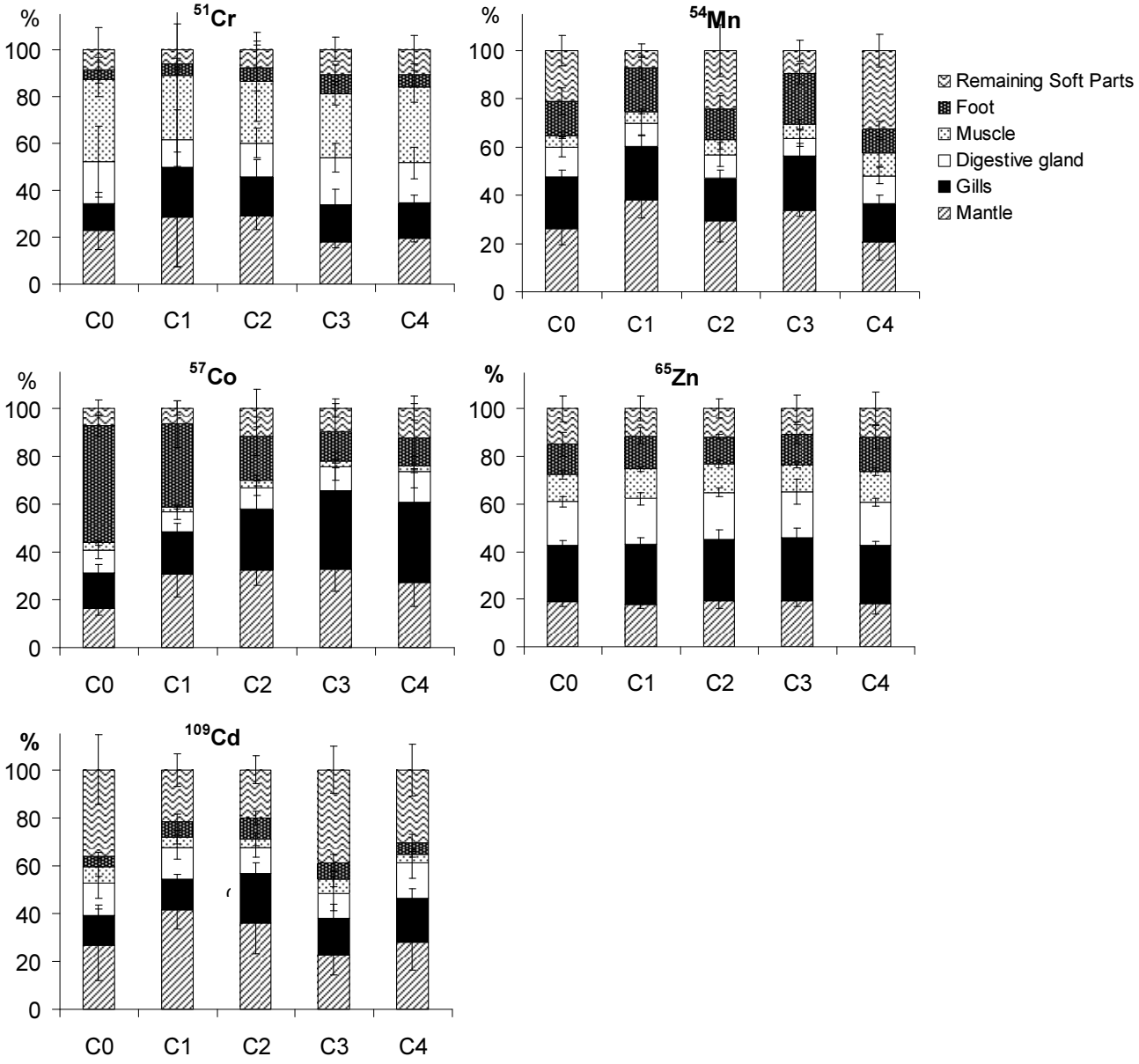


Figure 5. ***Gafrarium tumidum*****. Radiotracer distribution (mean % ± SD, n = 6) among the different body compartments of the clams maintained for 21 d in uncontaminated seawater after a 14-d exposure to 5 increasing concentrations (C0-C4) of Cr, Mn, Co, Zn and Cd in seawater.**



III.2. OYSTER *I. ISOGNOMON*

Whole-body uptake of ^{54}Mn, ^{57}Co, ^{65}Zn and ^{109}Cd in *I. isognomon* were best fitted by a linear regression for all concentrations tested (R^2: 0.72-0.96 for Mn, 0.77-0.87 for Co, 0.74-0.8 for Zn and 0.80-0.86 for Cd) (Fig. 6, Table 4), whereas the uptake kinetics of ^{51}Cr and ^{73}As were more accurately described by an exponential model (R^2: 0.84-0.90 for Cr and 0.58-0.75 for As). The estimated CF_{ss} indicated that ^{51}Cr uptake efficiency is one order of magnitude higher than ^{73}As. The estimated uptake rate constants (k_u) indicated that the elements were concentrated according to the following order of decreasing rate: Zn > Mn, Co > Cd, Cr, As.

LMM analyses indicated that the uptake kinetics of ^{51}Cr, ^{54}Mn and ^{109}Cd did not significantly differ ($p_{SPSS} > 0.05$) on the range of concentrations tested. In contrast, exposure concentration in seawater significantly affected the uptake kinetics of ^{57}Co, ^{65}Zn and ^{73}As (p_{SPSS} = 0.03, 0.005 and 0.0001, respectively). However, when the range of concentration is reduced up to 23 ng Co l^{-1} and 700 ng Zn l^{-1}, oysters accumulated Co and Zn with similar uptake rate constant.

The radiotracer distribution at the end of exposure period (Fig. 7) indicated that the major part of the radiotracers was associated with visceral mass, except for ^{57}Co. This latter radiotracer was mainly present in the mantle. The element distribution among the body compartments of oysters were similar over the whole range of concentrations tested.

Examination of the subcellular distributions in visceral mass and gills of the oysters (Fig. 8) indicated that Cd and Mn in both visceral mass and gills and Cr in gills were mainly (up to 82 %) associated with the soluble fraction whereas Zn in both organs and Co in gills were mainly (up to 79 %) associated with the insoluble fraction. In all other cases, the elements were distributed equally between the insoluble and soluble fraction.

Table 4. Estimated uptake rate constant (k_u, d^{-1}) and concentration factor at steady state (CF_{ss}) of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in the oyster *Isognomon isognomon* exposed to five increasing dissolved concentration for 14 d.

L: Linear model: $CF_t = k_u\, t$

E: Exponential model: $CF_t = CF_{ss}\,(1-e^{-k_e\, t})$

ASE: asymptotic standard error; R^2: determination coefficient of the uptake kinetics

Isotopes	Model	Concentration	$k_u \pm$ ASE	$CF_{ss} \pm$ ASE	R^2
^{51}Cr	E	C0	7.6 ± 0.9^{d}	159.5 ± 67.0^{a}	0.84
	E	C1	7.7 ± 0.7^{d}	159.3 ± 49.7^{b}	0.89
	E	C2	6.4 ± 0.6^{d}	131.2 ± 40.3^{b}	0.90
	E	C3	7.8 ± 0.9^{d}	168.7 ± 103.0 *	0.84
	E	C4	7.8 ± 0.8^{d}	118.4 ± 0.1^{d}	0.86
^{54}Mn	L	C0	22.7 ± 0.6^{d}		0.90
	L	C1	21.6 ± 0.3^{d}		0.96
	L	C2	31.2 ± 1.2^{d}		0.78
	L	C3	22.7 ± 1.0^{d}		0.72
	L	C4	25.8 ± 1.0^{d}		0.79
^{57}Co	L	C0	26.0 ± 0.8^{d}		0.85
	L	C1	25.4 ± 0.9^{d}		0.77
	L	C2	26.5 ± 0.7^{d}		0.87
	L	C3	18.5 ± 0.5^{d}		0.87
	L	C4	18.4 ± 0.5^{d}		0.83
^{65}Zn	L	C0	148.5 ± 5.8^{d}		0.79
	L	C1	154.2 ± 5.5^{d}		0.82
	L	C2	131.0 ± 4.9^{d}		0.80
	L	C3	140.2 ± 6.5^{d}		0.74
	L	C4	96.2 ± 3.0^{d}		0.85
^{73}As	E	C0	5.4 ± 0.6^{d}	25.2 ± 1.7^{d}	0.74
	E	C1	7.4 ± 0.8^{d}	17.2 ± 0.7^{d}	0.75
	E	C2	5.8 ± 0.7^{d}	14.7 ± 0.7^{d}	0.66
	E	C3	5.5 ± 0.8^{d}	10.4 ± 0.5^{d}	0.58
^{109}Cd	L	C0	10.4 ± 0.4^{d}		0.81
	L	C1	8.5 ± 0.3^{d}		0.80
	L	C2	10.4 ± 0.4^{d}		0.81
	L	C3	8.2 ± 0.2^{d}		0.85
	L	C4	9.8 ± 0.3^{d}		0.86

Significance of the estimated parameters: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$, * $p > 0.05$

Figure 6. *Isognomon isognomon*. Influence of stable Cr, Mn, Co, Zn, As and Cd concentrations in seawater on whole-body uptake of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in oysters (mean concentration factor ± SD, n = 9).

Parameters and statistics of the uptake kinetics are given in Table 4.

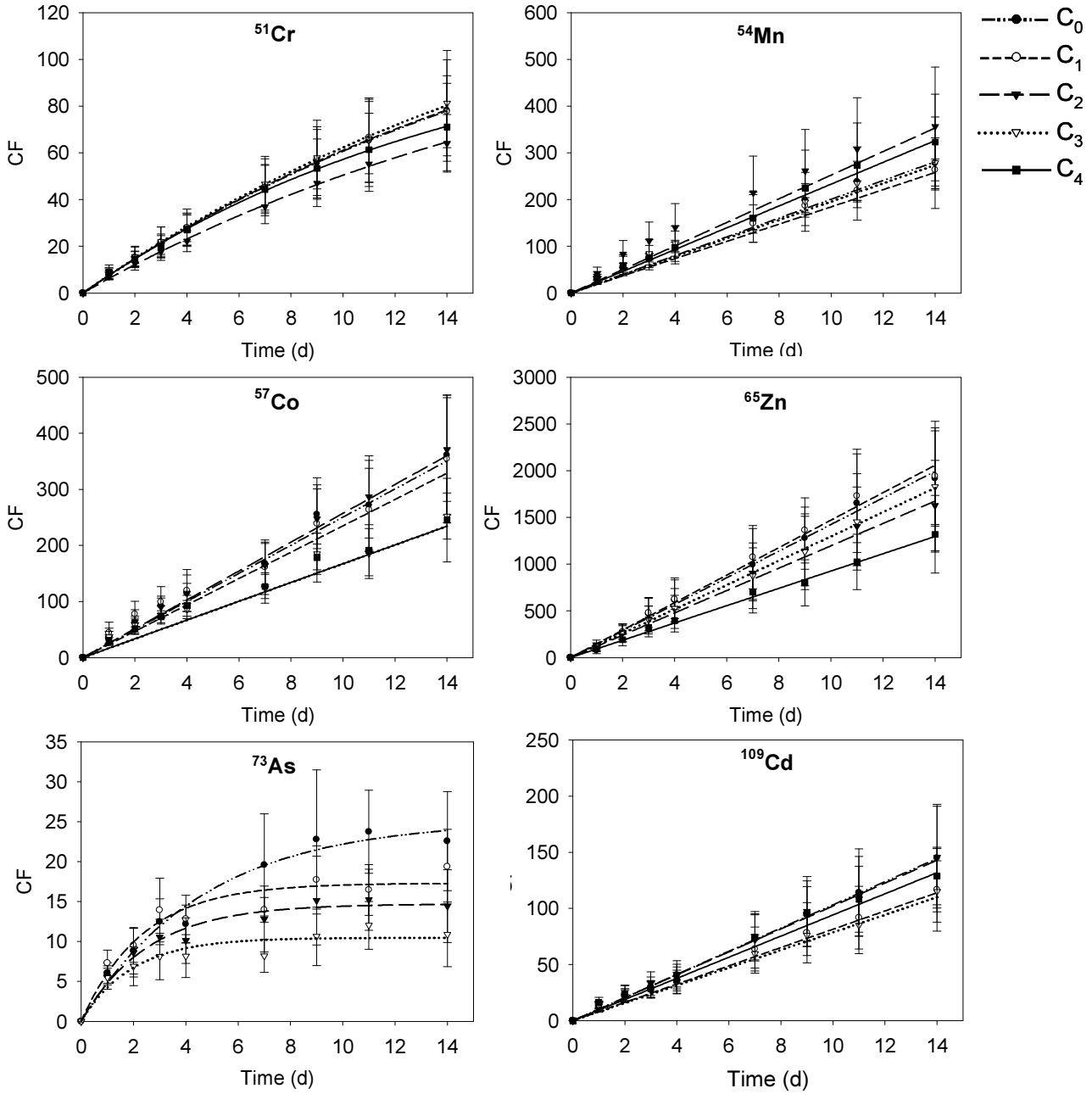


Figure 7. ***Isognomon isognomon*****. Radiotracer distribution (mean % ± SD, n = 3) among the different body compartments of the oysters exposed for 14 d to 5 increasing concentrations (C0-C4) of Cr, Mn, Co, Zn, As and Cd in seawater.**

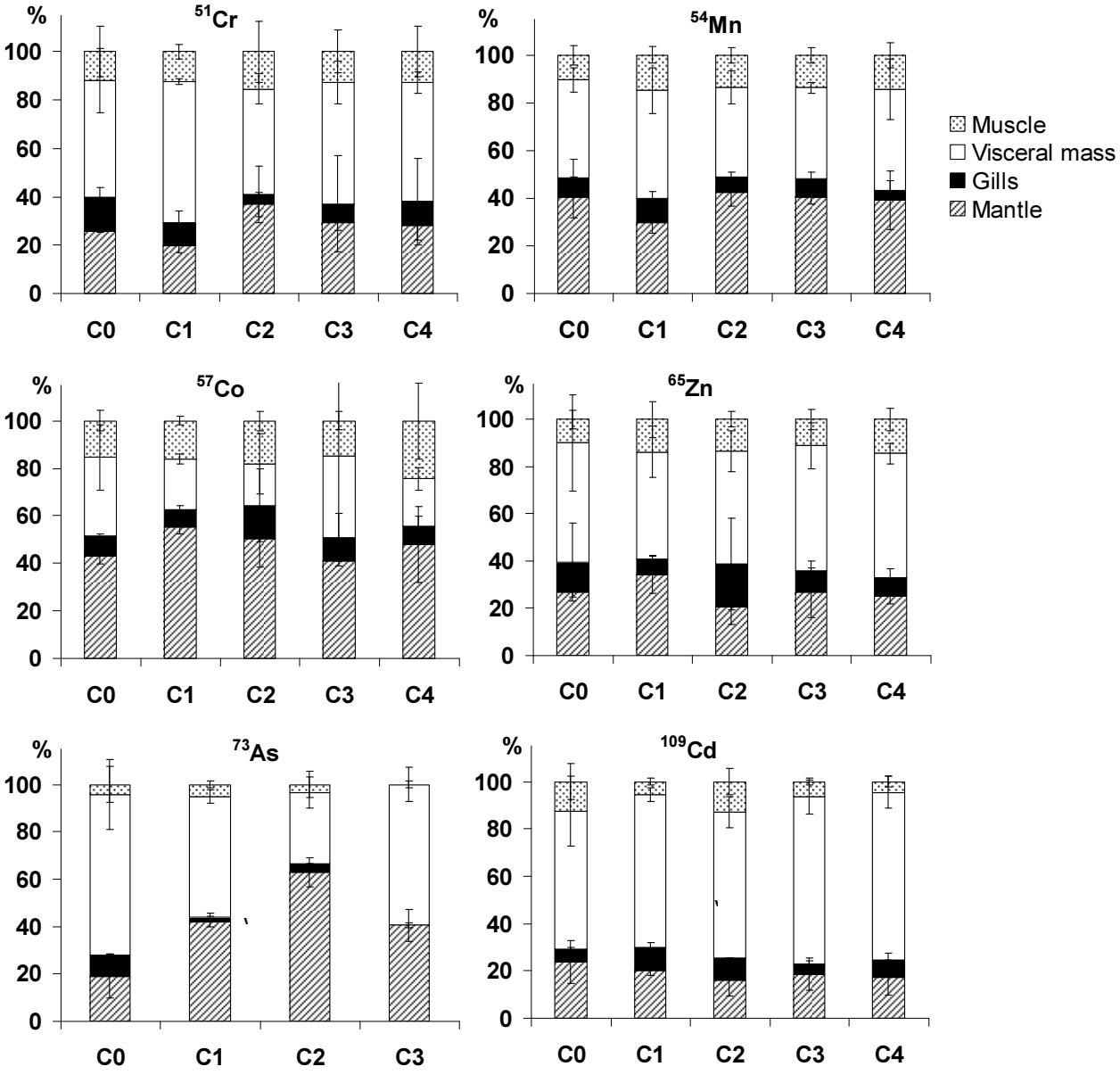
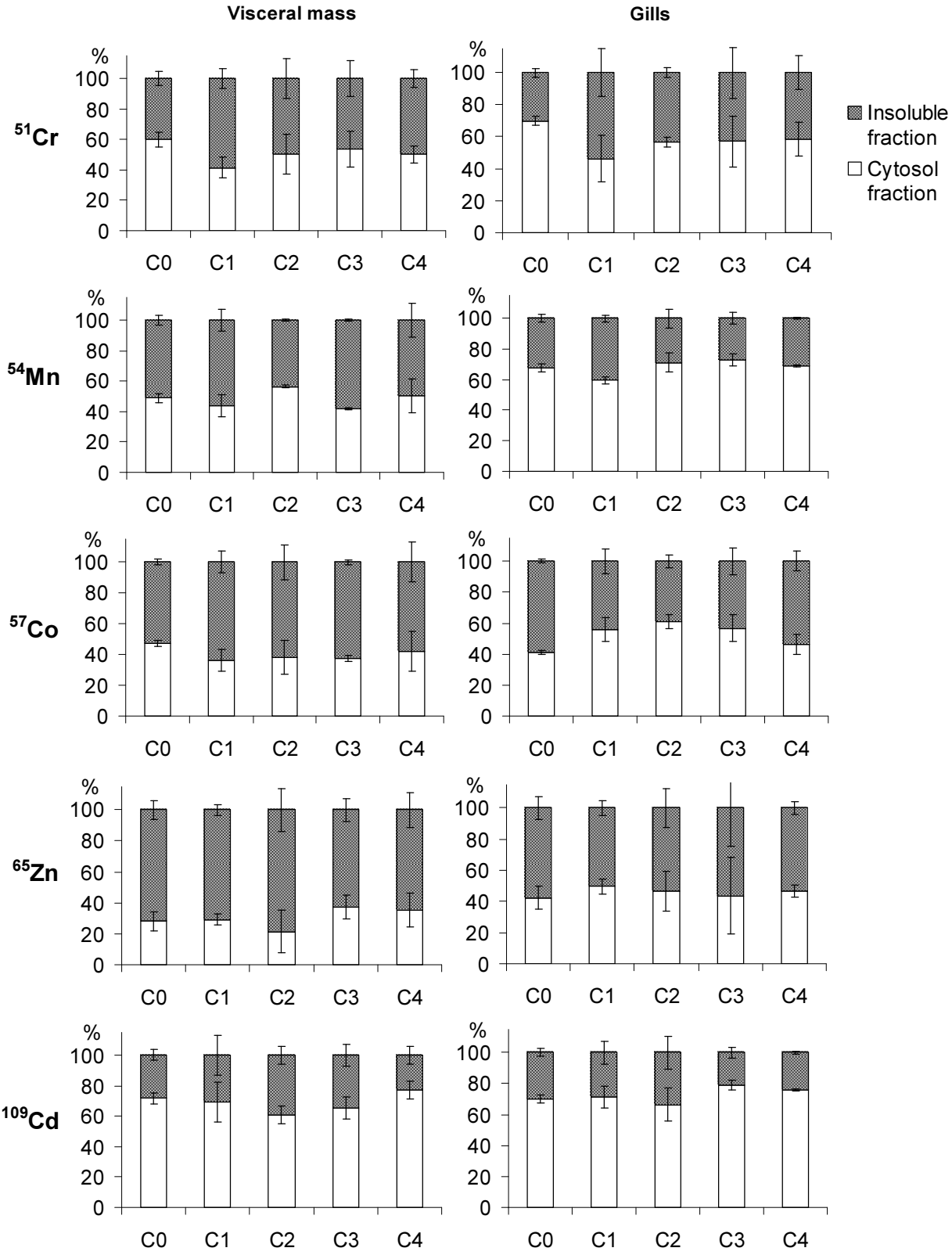


Figure 8. ***Isognomon isognomon*****. Subcellular distribution (mean % ± SD, n = 3) of radiotracers in the visceral mass and gills of the oysters exposed for 14 d to 5 increasing concentrations (C0-C4) of Cr, Mn, Co, Zn and Cd in seawater.**



Loss kinetics of ^{51}Cr, ^{54}Mn, ^{65}Zn and ^{109}Cd were best fitted by a single-component exponential model for all concentrations tested ($R^2 > 0.7$, except ^{65}Zn and ^{109}Cd for which $R^2 < 0.3$) (Fig. 9, Table 5). The $T_{b½}$ of radiotracers ranged from 27 to 41 d for ^{51}Cr, 26 to 28 d for ^{54}Mn, 91 to 96 d for ^{109}Cd, and to a time non different from the infinite for ^{65}Zn. Loss kinetics of the two other radiotracers (^{57}Co and ^{73}As) were more accurately described by a two-component exponential model, in which the short-lived component was characterized by a very short $T_{b½}$ (< 2 d). The long-lived components were characterized by $T_{b½}$ ranging from 17 to 23 d for ^{73}As and from 50 to 79 d for ^{57}Co. By order of decreasing retention time, tested elements can thus be ranked as follows: Zn > Cd > Co > Cr > Mn > As.

No significant difference ($p_{SPSS} > 0.05$) was observed among the loss kinetics determined along the concentration range tested, except for ^{65}Zn ($p_{SPSS} = 0.005$).

Comparison of the radiotracer distribution at the end of the exposure (Fig. 7) and depuration (Fig. 10) periods indicated that the relative distribution of all radiotracers in oysters remained quite constant throughout the experiment, except for ^{57}Co. With the exception of this latter element, the loss of radiotracers from the different body compartments could thus occur at similar rates. The distribution of ^{57}Co among the different body compartments indicated that a higher proportion of this element was associated with the visceral mass at the end of the depuration compared to the one observed at the end of the exposure period. This suggests that the visceral mass of oysters retains ^{57}Co more efficiently than all the other compartments.

Table 5. Parameters of the loss kinetics of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in the oyster *Isognomon isognomon* previously exposed for 14 d to radiotracers via seawater.

S: Single-component loss exponential model: $A_t = A_0 e^{-k_e t}$;

T: Two-component loss exponential model: $A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t}$

A_0 (%): Absorption efficiency, k_e (d^{-1}): loss rate constant and $T_{b½}$ (d): biological half life (when loss kinetics followed a T model, only A_{0l}, k_{el} and $T_{b½l}$ are indicated)

ASE: asymptotic standard error; R^2: determination coefficient of the loss kinetics

Isotopes	Model	Concentration	A_0 ± ASE	k_e ± ASE	$T_{b½}$ ± ASE	R^2
^{51}Cr	S	C0	94.6 ± 1.4 ^{d}	0.025 ± 0.002 ^{d}	27.8 ± 1.9	0.80
	S	C1	92.8 ± 1.3 ^{d}	0.024 ± 0.001 ^{d}	28.9 ± 1.8	0.81
	S	C2	94.6 ± 1.6 ^{d}	0.022 ± 0.002 ^{d}	31.6 ± 2.7	0.70
	S	C3	92.3 ± 2.2 ^{d}	0.025 ± 0.003 ^{d}	27.3 ± 2.9	0.59
	S	C4	92.1 ± 1.3 ^{d}	0.017 ± 0.002 ^{d}	40.8 ± 3.6	0.66
^{54}Mn	S	C0	97.2 ± 1.1 ^{d}	0.025 ± 0.001 ^{d}	28.1 ± 1.4	0.87
	S	C1	96.1 ± 0.9 ^{d}	0.027 ± 0.001 ^{d}	25.7 ± 1.0	0.91
	S	C2	98.4 ± 0.9 ^{d}	0.027 ± 0.001 ^{d}	25.5 ± 0.9	0.92
	S	C3	97.1 ± 1.7 ^{d}	0.025 ± 0.002 ^{d}	28.3 ± 2.2	0.73
	S	C4	98.3 ± 1.4 ^{d}	0.025 ± 0.002 ^{d}	27.7 ± 1.7	0.81
^{57}Co	S	C0	85.5 ± 3.6 ^{d}	0.012 ± 0.003 ^{c}	58.4 ± 15.8	0.62
	S	C1	84.4 ± 2.5 ^{d}	0.014 ± 0.002 ^{d}	49.7 ± 8.5	0.70
	S	C2	78.7 ± 3.9 ^{d}	0.009 ± 0.004 ^{a}	77.0 ± 30.9	0.69
	S	C3	74.6 ± 4.0 ^{d}	0.013 ± 0.004 ^{b}	52.1 ± 15.5	0.71
	S	C4	81.8 ± 4.4 ^{d}	0.009 ± 0.004 ^{a}	79.0 ± 34.3	0.55
^{65}Zn	T	C0	99.2 ± 1.1 ^{d}	0.004 ± 0.001 ^{c}	176 ± 47	0.17
	T	C1	99.2 ± 0.6 ^{d}	0.001 ± 0.001 *	772 ± 486	0.04
	T	C2	99.4 ± 0.8 ^{d}	0.002 ± 0.001 ^{b}	335 ± 119	0.10
	T	C3	96.0 ± 1.3 ^{d}	0.001 ± 0.001 *	686 ± 831	0.10
	T	C4	95.1 ± 1.1 ^{d}	0.003 ± 0.001 ^{a}	242 ± 92	0.09
^{73}As	T	C0	85.7 ± 5.9 ^{d}	0.039 ± 0.006 ^{d}	17.9 ± 2.7	0.78
	T	C1	80.5 ± 11.7 ^{d}	0.042 ± 0.010 ^{d}	16.5 ± 3.9	0.82
	T	C2	85.9 ± 6.4 ^{d}	0.038 ± 0.006 ^{d}	18.4 ± 2.9	0.72
	T	C3	83.7 ± 5.0 ^{d}	0.030 ± 0.005 ^{d}	23.4 ± 3.6	0.73
^{109}Cd	T	C0	90.9 ± 2.0 ^{d}	0.008 ± 0.002 ^{c}	90.9 ± 2.0	0.22
	T	C1	94.2 ± 1.6 ^{d}	0.006 ± 0.002 ^{c}	94.2 ± 1.6	0.18
	T	C2	90.6 ± 1.9 ^{d}	0.006 ± 0.002 ^{b}	90.6 ± 1.9	0.15
	T	C3	92.7 ± 2.1 ^{d}	0.009 ± 0.002 ^{d}	92.7 ± 2.1	0.25
	T	C4	95.5 ± 1.8 ^{d}	0.005 ± 0.002 ^{c}	95.5 ± 1.8	0.15

Significance of the estimated parameters: ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$, ^{d} $p < 0.0001$, * $p > 0.05$

Figure 9. *Isognomon isognomon*. Influence of stable Cr, Mn, Co, Zn, As and Cd concentrations in seawater on whole-body loss of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{73}As and ^{109}Cd in oysters (mean % remaining activities, A (%), ± SD, n = 6).

Parameters and statistics of the loss kinetics are given in Table 5.

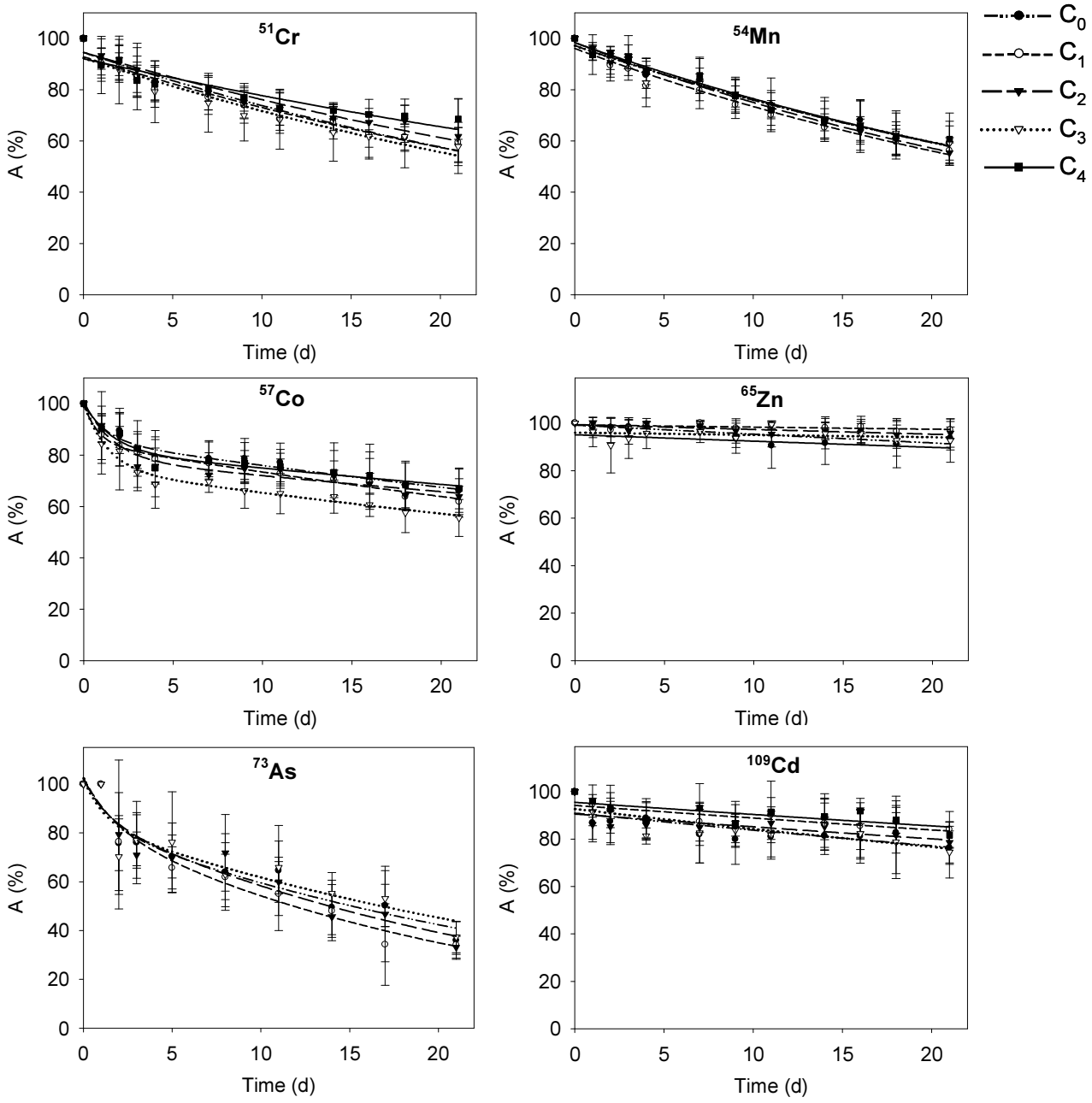
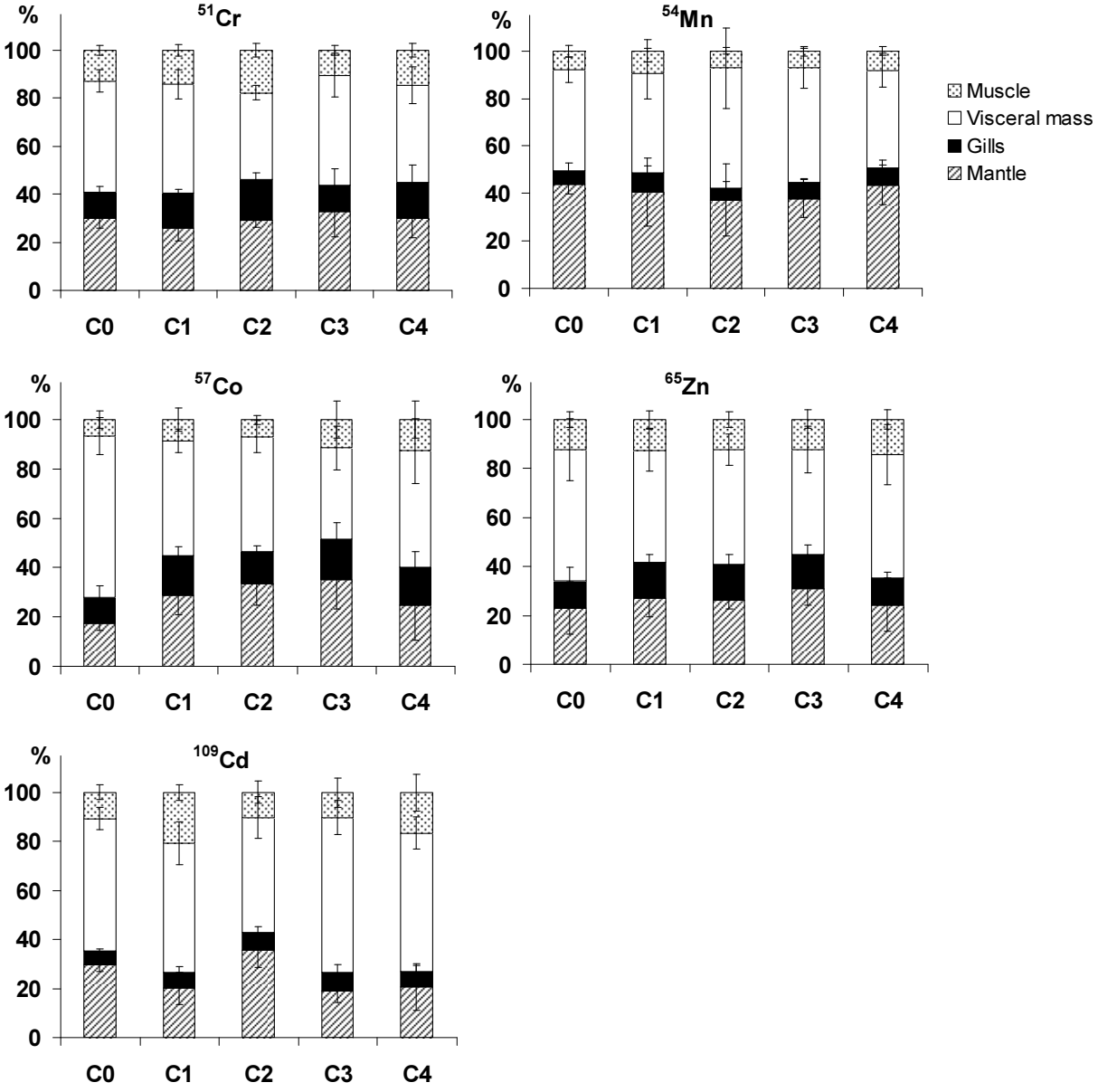


Figure 10. ***Isognomon isognomon*****. Radiotracer distribution (mean % ± SD, n = 6) among the different body compartments of the oysters maintained for 21 d in uncontaminated seawater after a 14-d exposure to 5 increasing concentrations (C0-C4) of Cr, Mn, Co, Zn and Cd in seawater.**



IV. DISCUSSION

Contaminant concentrations in bioindicator tissues should reflect, according to a simple relationship, those occurring in the surrounding environment (Phillips 1976a, 1990). This criterion is essential since at least some organisms (e.g. crustacean) are know to be able to control their internal concentrations in one or several metals (White & Rainbow 1984; Depledge & Rainbow 1990). As a result, internal concentrations in these organisms do not reflect those occurring in the environment, and they can thus not be used as bioindicator. Although most relevant, the relationship between internal and external concentration has been rarely assessed in the process of characterizing of bioindicator species. Indeed, selection of bioindicators is generally more depending on the species available in the studied areas rather than on their actual reliability to reflect contamination status of the environment (Phillips 1990; Warnau 1996). Since this can lead to weird ecotoxicological interpretation of metal levels recorded in organisms, influences of contamination levels in the environment on the bioconcentration capacities of the potential bioindicators actually need to be investigated. This is particularly true in the New Caledonian lagoon, where marine organisms may be exposed to a wide range of dissolved concentrations (> 2 orders of magnitude) according to their location in the lagoon (Fernandez et al. 2002a). Ideal bioindicator would bioconcentrate elements following a proportional relationship with ambient dissolved element concentration, resulting in a concentration factor CF that would remain constant.

When element concentration in seawater increases, 3 different trends can be observed: (1) CF remains constant over the whole range of element concentrations; (2) CF remains constant until a threshold concentration is reached and then CF decreases; (3) CF decreases continuously over the range of concentration considered (Bouquegneau & Joiris 1988; Warnau et al. 1997). Although two of these trends were present in this work, the most common relationships observed was a constant CF or CF_{ss} (when observed uptake kinetics reached saturation) over the range of dissolved concentrations tested. Indeed, the whole-body uptake kinetics of Cr, Mn and Cd were not affected by dissolved element concentration ($p_{SPSS} > 0.05$). This indicates that bioconcentration of these elements in clams and oysters is directly proportional to the element concentration in surrounding seawater. Similar results have been reported for other organisms, e.g. Cd, Cu and Pd in the mussel *Perna viridis* (Chan 1988), Cd in *Mytilus edulis* (Poulsen et al. 1982; Fisher 1988) and Cd in the echinoid *Paracentrotus lividus* (Warnau et al. 1997).

In contrast, uptake kinetics of As, Co and Zn were affected by the element concentration in seawater in both bivalves. When the As concentration increased in seawater, the CF_{ss} in oysters decreased regularly from 25.2 to 10.4. Such a trend indicated that *I. isognomon* can hardly be used as a direct quantitative bioindicator of ambient dissolved levels of As. The present study indicated that the oysters *I. isognomon* displayed a limited capacity to accumulate As from seawater ($CF_{SS} < 25.2$), an observation supported by previous studies on the mussel *Mytilus galloprovincialis* (Ünlü & Fowler 1979). However, very high As concentrations have been measured in the field in *I. isognomon* (77 µg As g^{-1}dry wt) and *G. tumidum* (441 µg As g^{-1}dry wt) (Hédouin et al. submitted-a), which indicate that As is actually taken up by these bivalves. This suggests that As is most probably bioaccumulated through another pathway than dissolved phase, which would support the hypothesis of Sanders, Osman & Riedel (1989) who proposed the dietary pathway as the main source of As in marine organisms.

CF of Co in both bivalves remained constant up to 23 ng l^{-1} added Co in seawater then CF decreased when Co concentration increased. Statistical analyses indicated that this decreasing trend would be due to a decrease in uptake rate in oysters and to an increase in loss rate in clams. This suggests the presence of regulatory protective processes, which reduce the uptake (in oysters) or increase the depuration (in clams) efficiency of these elements. Bioconcentration of Zn in the oysters displayed the same trend as Co; although the threshold value was much higher : a net decrease in k_u has been observed only for added Zn concentration higher than 700 ng Zn l^{-1}. Similar patterns have been reported for Cd in the asteroid *Asterias rubens* (Bjerregaard 1988) and As in the mussel *Mytilus galloprovincialis* (Ünlü & Fowler 1979). Our results indicate that *G. tumidum* and *I. isognomon* could thus be used as bioindicator species of Co and Zn contamination only over a restricted range of dissolved concentrations (viz. our background concentrations + 23 ng added Co l^{-1} and 700 ng added Zn l^{-1}). Dissolved concentrations up to 20 ng Co l^{-1} and 1500 ng Zn l^{-1} have been reported in highly contaminated coastal zones of the New Caledonia lagoon (Goro-Nickel 2004). This means that our experimental concentration range up to 23 ng added Co l^{-1} covered the whole field one. Regarding Zn, the background dissolved concentration in the natural seawater used in our experiments ranged between 328 and 590 ng Zn l^{-1} (Ferrier-Pagès et al. 2005). This indicates that bivalves took up Zn proportionally to the ambient dissolved concentration up to 1028 to 1290 ng Zn l^{-1} (background Zn concentration in experimental

seawater + Zn added concentration), which almost matches the Zn concentration range encountered in the New Caledonian lagoon.

Obtaining information about bioconcentration capacities of different organs is of major interest since some organs may be more sensitive and more specifically able to inform about differences in element contamination in the environment (e.g. Osuna Lopez et al. 1990). In this study, gills and digestive organs were the main target compartments for waterborne element in both *I. isognomon* and *G. tumidum*. The high concentration capacity observed in gills is usually explained by their large exchange surface and high pumping rate in both bivalves (Arifin & Bendell-Young 2000). However, elements were also found to be largely distributed in digestive organs (digestive gland in clams and visceral mass in oysters). Digestive organs are known to highly accumulate dissolved elements probably due to the presence of complexing agents and their involvement in storage and detoxification processes (e.g. George & Coombs 1977; Bustamante et al. 2002).

The efflux rate of all elements in both studied bivalves was generally not affected by the previous exposure concentration in seawater ($p_{SPSS} > 0.05$), except for Zn in oysters. Similar results have been found for Cd in the sea urchin *Paracentrotus lividus* (Warnau et al. 1997) and the green mussel *Perna viridis* (Blackmore & Wang 2002). For example, the efflux rate of Cd (0.007-0.012 d^{-1}) in green mussel *P. viridis* was not significantly different after 7-d exposure to 0 and 100 µg l^{-1} of added Cd. Although poorly realistic, the very high value of this latter exposure concentration indicated that this non-dependently should hold true for the whole range of concentration that can be encountered in the marine environment. Our study showed that all elements tested were efficiently retained in both bivalve species ($T_{b½} > 20$ d), suggesting that they are able to preserve information regarding their contamination history for a relatively long period of time (several weeks). This is particularly true in the case of Cd and Zn, for which the highest retention capacity has been observed and for which different mechanisms of adaptation can be proposed

The particularly high retention efficiency of Zn in *I. isognomon* (estimated $T_{b½}$ Zn = ∞) and its high uptake rate (k_u Zn = 150 d^{-1}) explain the capacity of oysters to store large amount of Zn in their tissues. This has been reported for several oyster species (e.g. Ruddell & Rains 1975; Engel & Brouwer 1982), and especially in the *Isognomon* genus (e.g. 4010 µg Zn g^{-1} in *I. alatus* from Dominican Republic, Sbriz et al. 1998; 12163 µg Zn g^{-1} from Guadeloupe, RNO-Antilles unpublished work). Furthermore, subcellular distribution investigations indicated that Zn was mainly associated with the non-cytosolic cellular fraction, suggesting

that a large proportion of Zn in *I. isognomon* could be stored in insoluble granules (George & Pirie 1980; Langston et al. 1998). Immobilisation of Zn in insoluble granules has already been found in other bivalves, such as the blue mussel *Mytilus edulis* (Langston et al. 1998) and *Perna viridis* (Blackmore & Wang 2002).

In the case of Cd, the very efficient retention observed here ($T_{b½}$ > 3 months) is consistent with the observations reported in *Mytilus edulis* ($T_{b½}$ = 67 to 190 d, Borchardt 1983; Wang et al. 1996) and *Mytilus galloprovincialis* ($T_{b½}$ = 1155 d) (Fowler & Benayoun 1974). Such a high retention capacity of non-essential element is generally thought to involve the induction of detoxification mechanism(s) allowing their storage under non-toxic form (Jeantet et al. 1985; Martoja & Martin 1985). It is well known that cytosolic proteins such as metallothioneins play a major role in binding Cd in many organisms (e.g. Roesijadi 1992; Mason & Jenkins 1995) and this could be the case in the clam *G. tumidum* and the oyster *I. isognomon*. Indeed, our results of subcellular distribution indicate that the cytosolic fraction in the digestive gland and gills of both clams and oysters contained a remarkably high proportion of the total load of Cd (70 to 95 %). Among the contaminants investigated in this study, Cd is one of the most toxic elements and is thus of major concern. Recent works have shown that subcellular distribution of an element in an organisms may affect its assimilation efficiency by the predators (Wallace et al. 2003; Wallace & Luoma 2003). Indeed, element bound to granules are thought to be less bioavailable to higher trophic levels (e.g. Nott & Nicolaidou 1990; 1994), whereas element occurring in the cytosolic fraction would represent an easily transferable pool to predators (Ettajani et al. 2001). Since the clam *G. tumidum* is a seafood product widely consumed in New Caledonia, it arises from above that Cd could be easily transferred to human. Therefore, special attention should be paid to Cd contamination in the clam *G. tumidum*.

V. CONCLUSIONS

The general constancy of uptake and loss rates of both bivalves over a large range of dissolved element concentrations confirms that element concentrations measured in clams and oysters would directly reflect the degree of contamination occurring in seawater. Furthermore, except for Co, distribution of elements among body compartments and subcellular fractions were not influenced by an increase in element concentration in seawater. Bioconcentration capacities (k_u and $T_{b½}$) of both bivalves indicate that clams and oysters are able to rapidly

detect dissolved contamination and to preserve the information on contamination event over long periods of time (several weeks). The results of this study bear out the potential usefulness of *G. tumidum* and *I. isognomon* as bioindicator species of dissolved element contamination in the framework of survey and monitoring studies. The range of dissolved concentrations tested in this work cover those encountered in the New Caledonian lagoon. Indeed, with the few limitations indicated previously (As, and to a lesser extend, Zn), the concentration of the elements in both species investigated would be generally proportional the dissolved concentrations. Both bivalve species are thus very promising bioindicators for tropical marine environment. Nevertheless, care should be taken when applying these results to other situations. Indeed, since some concentration effects were demonstrated, results may only be valid for the range of concentration considered here. Furthermore, although dissolved contamination (via lixiviation) is the main concern in the New Caledonia lagoon, several studies have shown that food may be a very important uptake pathway for metals (Wang et al. 1996; Hédouin et al. to be submitted-a, to be submitted-b). Therefore, food-related influence on contaminant bioaccumulation in these bioindicator species should be investigated in order to complement the results of this study.

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CHAPITRE 6

Lobophora variegata, une espèce bioindicatrice pour la surveillance de la contamination métallique dans l'environnement marin tropical : caractérisation expérimentale à l'aide des radiotraceurs

La bioconcentration des métaux dans l'algue brune *Lobophora variegata* a été étudiée afin d'évaluer sa valeur en tant que bioindicateur de la contamination métallique d'origine minière dans le lagon de Nouvelle-Calédonie. Les cinétiques d'accumulation et d'élimination de six métaux (Cr Mn, Co, Ni, Zn et Cd) ont été déterminées en exposant les algues à des concentrations métalliques dissoutes croissantes et réalistes à l'aide des techniques de radiotraçage. Les expériences ont été effectuées afin de déterminer l'influence des différentes concentrations dissoutes sur les capacités de bioconcentration et de rétention des métaux dans l'algue. Les résultats indiquent que l'algue incorpore le Cr, Co, Ni, Zn, et le Cd proportionnellement aux concentrations dissoutes ambiantes sur toute la gamme de concentrations testée (trois ordres de grandeur). En revanche, le Mn est accumulé de manière proportionnelle sur une gamme de concentration dissoute de 2 ordres de grandeurs, son efficacité d'accumulation diminue légèrement aux fortes concentrations testées.

De manière générale, *L. variegata* apparaît comme une excellente espèce bioindicatrice capables d'accumuler rapidement les métaux et de fournir des informations quantitatives pertinentes sur les niveaux de contamination présents dans l'environnement environnant.

En outre, de part sa large répartition géographique, *L. variegata* pourrait être utilisée comme un bioindicateur fiable pour surveiller la contamination métallique dans d'autres régions tropicales.

Lobophora variegata, a bioindicator species for surveying metal contamination in tropical marine environment: experimental characterisation using radiotracer techniques

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To be submitted

ABSTRACT. Metal bioconcentration was investigated in the brown alga *Lobophora variegata* in order to assess its value as bioindicator to survey and monitor mining-originating metal contamination in the New Caledonia lagoon. Uptake and loss kinetics of six metals (Cr, Mn, Co, Ni, Zn and Cd) were determined during experimental exposures to increasing, environmental realistic concentrations of each metal using highly sensitive radiotracer techniques. The experiments were carried out in order to assess the possible influence of varying dissolved concentrations on metal bioconcentration and retention capacities in the alga. Results indicate that the alga takes up Cr, Co, Ni, Zn, and Cd in direct proportion to their ambient dissolved concentrations over the whole range of concentration tested (three orders of magnitude). In contrast, Mn was taken up proportionally to its dissolved concentrations over a range of 2 orders of magnitude, then its accumulation efficiency slightly decreased. Overall, *L. variegata* appears as an excellent bioindicator species that shows a rapid response time in metal uptake and has a suitable potential to furnish valuable quantitative information on the contamination levels occurring in the surrounding environment. Furthermore, due to its wide geographical distribution, *L. variegata* could be considered as a relevant candidate bioindicator for surveying metal contamination in many other tropical areas.

Keywords: Brown alga, Tropic, Metal, Bioconcentration, Bioindicator, Radiotracer techniques

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I. INTRODUCTION

The main economic resources of New Caledonia are derived from nickel exploitation. The land-based open-cast mining activities inevitably result in direct and indirect metal contamination of the surrounding environments and in particular in the lagoon waters (Ambatsian et al. 1997; Labrosse et al. 2000). Although these metal inputs constitute a threat to the local, highly diversified coastal marine ecosystems, very little information is available regarding levels of contamination and possible impairments of the New Caledonian lagoon (Labrosse et al. 2000).

Among the common approaches used to study environmental contamination, the use of bioindicator species has proved to be a valuable and informative tool. In the marine environment, bivalves are the most widely used bioindicator species and were e.g., successfully used in the implementation of large scale monitoring programme such as the US Mussel Watch program (Goldberg et al. 1983). Although less commonly used, marine macrophytes and, in particular seaweeds, are well documented for their ability to concentrate contaminants (Bryan & Hummerstone 1973; Phillips 1994b; Warnau et al. 1996a).

According to a recent study, the brown alga *Lobophora variegata* has been identified as a valuable potential boindicator species to be used in the New Caledonian lagoon (Breau 2003; Hédouin et al. submitted-a). Indeed, this alga shows a good capacity to bioaccumulate metals from its surrounding environment, is abundant and easy to collect. Although the alga meets most of the criteria that should fulfil a bioindicator species (Phillips 1994b), no information is available on one of the most important prerequisite: the existence of a simple relationship between metal concentrations in organisms and those in seawater (Phillips 1977b). For this reason, the influence of ambient metal concentrations on their bioconcentration and depuration in *L. variegata* was investigated in order to further assess its value as a reliable sentinel species.

II. MATERIALS AND METHODS

II.1. SAMPLING

Brown algae *Lobophora variegata* (Lamouroux) Womersley were collected by SCUBA diving in the South Western lagoon of New Caledonia (Maa Bay) in October 2003. This site

is considered as a reference site in terms of metal contamination (Breau 2003; Hédouin et al. submitted-a). Algae were then shipped to IAEA-MEL premises in Monaco and were acclimated for 1 month before experiments (open circuit aquarium; seawater renewal: 50% hr^{-1}, salinity: 36 ± 1 p.s.u.; T°: 25 ± 1°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs)

II.2. STABLE ELEMENTS AND RADIOTRACERS

Investigated elements (Cr, Mn, Co, Ni, Zn and Cd) were introduced into the experimental microcosms as both stable elements and their corresponding radiotracers (five γ-emitting tracers: ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn ^{109}Cd, and one β-emitting isotope:^{63}Ni) to allow measuring elements with high sensitivity. Stable elements were introduced as HNO_3 salts (Merck, synthesis quality). Radiotracers of high specific activity were purchased from Amersham, UK (^{51}Cr as Na_2CrO_4, $T_{½}$ = 27.7 d; ^{57}Co as $CoCl_2$, $T_{½}$ = 271.8 d; ^{63}Ni as $Ni(NO_3)_2$, $T_{½}$ = 100 yr), Isotope Product Lab., USA (^{109}Cd as $CdCl_2$, $T_{½}$ = 426.6 d; ^{65}Zn as $ZnCl_2$; $T_{½}$ = 243.9 d; ^{54}Mn as $MnCl_2$, $T_{½}$ = 312.2 d).

II.3. EXPERIMENTAL PROCEDURE

For each element tested, five batches (n = 5 for Cr, Co, Mn, Zn, Cd ; n=48 for Ni) of five *L. variegata* thallia (average weight = 1.7 ± 0.7 g wet wt; 0.16 ± 0.03 g dry wt) were placed in five aquaria containing 20 l of natural sea water (closed circuit aquarium; salinity: 36 ± 1 p.s.u.; T°: 25 ± 1°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). In order to allow for alga identification in γ-emitting tracer exposure experiments, each individual seaweed thallium used was kept into a cylindrical container (80 mm x 50 mm) covered above and below with 300 µm-size mesh net (to allow for free water circulation). Algae were then exposed for 14 d to five increasing added concentrations of a given element, up to 1250 ng Cr l^{-1}, 998 ng Co l^{-1}, 1250 ng Mn l^{-1}, 1400 ng Ni l^{-1}, 1750 ng Zn l^{-1} and 250 ng Cd l^{-1} (Table 1). The concentration ranges were selected in order to cover those actually encountered in the New Caledonia lagoon waters (Fernandez et al. 2002a). The added concentrations were realized using increasing amount of the stable element and a fixed activity of the corresponding radiotracer: ^{51}Cr (1.5 kBq l^{-1}), ^{54}Mn (0.5 kBq l^{-1}), ^{57}Co (0.5 kBq l^{-1}), ^{63}Ni (1 kBq l^{-1}), ^{65}Zn (0.5 kBq l^{-1}) and ^{109}Cd (1 kBq l^{-1}). In terms of stable element additions, these activities corresponded to Cr (1.39 10^{-10} g l^{-1}), Mn (3.55 10^{-10} g l^{-1}), Co (2.75 10^{-11} g l^{-1}), Ni (4.2 10^{-9} g l^{-1}), Zn (5.99 10^{-9} g l^{-1}) and Cd (4.84 10^{-11} g l^{-1}). No change in pH was detectable after radiotracer and element additions. Seawater and spikes were renewed daily for 5 days, then every second day in order

to keep exposure concentrations as constant as possible. Concentrations of the element in seawater were checked daily, and before and after each seawater renewal.

After 14d of exposure, non-contaminating conditions were restored (open circuit aquarium; seawater renewal: 50% hr^{-1}, salinity: 36 ± 1 p.s.u.; T°: 25 ± 1°C; pH: 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) in order to follow the loss kinetics of the radiotracers from the algae. Depuration period lasted for 21 d (Cr, Co, Mn, Zn, Cd) or 28d (Ni).

At different time intervals during the exposure and subsequent depuration period, seaweeds were collected and radioanalysed to determine radiotracer uptake and loss biokinetics, respectively. Seaweeds (n=5) exposed to γ-emitting tracers were counted alive (see below) and then replaced in their respective aquarium. For algae exposed to ^{63}Ni, thallia (n = 3) were collected at each sampling time and prepared for LSC analysis (see below).

II.4. RADIOANALYSIS

Due the methodological specificities of γ- and β-counting, γ-emitting tracers (^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{109}Cd) were radiolanalysed on a wet wt basis whereas β-emitting ^{63}Ni was analysed on a dry wt basis. Therefore, in order to allow for direct comparison with the other metals, all Ni-related results were further expressed on a wet wt basis using the measured wet: dry wt ratio of 3.333.

Radioactivity of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn and ^{109}Cd was measured using a high-resolution γ-spectrometer system composed of 4 Germanium -N or P type- detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser (Intergamma, Intertechnique). The radioactivity of samples was determined by comparison with standards of known activities of appropriate geometry. All measurements were corrected for counting efficiency, background and radioactive decay. Counting time was adapted to obtain counting rates with propagated errors less than 5%.

Radioanalyses of ^{63}Ni-exposed algae were performed using a 1600 TR Packard Liquid Scintillation Analyzer (destructive analyses method). Seawater samples (2 ml) containing radiotracer were directly transferred to 20 ml glass scintillation vials (Packard) and mixed with 10 ml of scintillation liquid (Ultima Gold®, Packard). Biota samples (thallia) were first dried at 60°C until constant weight, then digested for one week at room temperature of 50°C with 1 ml of Soluene® (Packard) for 100 mg dry weight tissues, and then mixed with scintillation liquid (Hionic Fluor®, Packard) in the proportion 1:5 volume Soluene® and

Hionic Fluor®. Counting time was adapted to obtain a propagated counting error less than 5 % (maximal counting duration 2 hrs). The radioactivity was determined by comparison with standards of known activities and measurements were corrected for counting efficiency, physical radioactive decay and quenching effects.

II.5. DATA TREATMENT

Uptake kinetics of the six investigated radiotracers were expressed in terms of concentration factor (CF, ratio between activity of the radiotracer in the organism -Bq g^{-1} wet wt and time-integrated activity of the radiotracer in seawater -Bq g^{-1}-). Radiotracer uptake kinetics were described using either a simple linear regression model (eq.1) or, if the observed kinetics tended to reach a steady-state equilibrium, using a saturation exponential kinetic model (eq.2):

$CF_t = k_u\, t$ (eq. 1)

$CF_t = CF_{ss}\,(1- e^{-k_e\, t})$ (eq. 2)

where CF_t and CF_{ss} are the concentration factors at time t (d) and at steady state, and k_u and k_e the uptake and loss rate constants (d^{-1}), respectively (Whicker & Schultz 1982). Linearity of the uptake kinetics was tested by a linearity test for regression with replication Zar 1996. Model constants (CF_{ss}, k_u and k_e) and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation using the non-linear curve-fitting routines in the Statistica® 6 software.

Loss kinetics of the radiotracers were expressed as the percentage of remaining radioactivity (radioactivity at time t divided by the initial radioactivity measured in the organism at the beginning of the depuration period). The loss kinetics of radiotracers were best fitted by either a single-component (eq.3) or a double-component (eq.4) exponential equation:

$A_t = A_0\, k_e\, t$ (eq. 3)

$A_t = A_{0s}\, e^{-k_{es}\, t} + A_{0l}\, e^{-k_{el}\, t}$ (eq. 4)

Where A_t, A_o are the remaining activities (%) at time t (d) and t = 0, respectively, k_e is the depuration rate constant (d^{-1}) and 's' and 'l' are the subscripts for the short-lived and long-lived component, respectively. For each exponential component (s and l), a biological half-life can be calculated ($T_{b½s}$ and $T_{b½l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b½} = \ln 2/k_e$ (Whicker & Schultz 1982).

In order to test the differences among exposure concentrations investigated, the estimated kinetic parameters (k_u, CF_{ss}, k_{es}, k_{el}) were plotted against the concentration of total element (stable + radioactive) in seawater and fitted using simple linear regression.

In the case of the γ-emitting tracers tested (^{51}Cr, ^{54}Mn, ^{57}Co, ^{65}Zn, ^{109}Cd) for which there was no time independence, differences among uptake and loss kinetics of each radiotracer along the concentration range were tested using the Linear Mixed Models (LMM) procedure implemented in SPSS v12.0 software. This procedure expands the general linear model so that the error terms and random effects are permitted to exhibit correlated and non-constant variability. The LMM, therefore, provides the flexibility to model not only the mean of a response variable, but its covariance structure as well. Time was specified as the repeated effect and the total dissolved concentrations (stable + radioactive) of Cr, Co, Mn, Zn and Cd as the fixed effect.

The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

Figure 1 shows the uptake kinetics of the 6 investigated metals in *Lobophora variegata* over the tested concentration ranges. Cr, Co, Zn, and Cd were taken up according to linear uptake kinetics ($R^2 > 0.65$) whereas Mn and Ni uptake were best described by a saturation model ($R^2 > 0.80$) for each concentration tested (Table 1). All metals were readily incorporated in *L. variegata* at each tested concentration, with value of uptake rate constant ranging from 66 to 1023 d^{-1}.

Linear Mix Model (LMM) analysis indicated that uptake kinetics of ^{51}Cr, ^{54}Mn, ^{57}Co and ^{65}Zn were not significantly different over the range of concentrations tested ($p_{SPSS} = 0.408$, 0.070, 0.292, 0.256, respectively). In contrast, for ^{109}Cd, exposure concentration significantly affected uptake kinetics in algae ($p_{SPSS} = 0.02$).

Figure 1. Influence of Cr, Mn, Co, Ni, Zn and Cd dissolved concentrations in seawater on whole body uptake kinetics of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{63}Ni, ^{65}Zn and ^{109}Cd in *Lobophora variegata* (concentration factor, CF, mean ± SD, n = 5, except for Ni, n = 3).

Parameters and statistics of the uptake biokinetics and exposure concentrations (C0-C4) are given in Table 1.

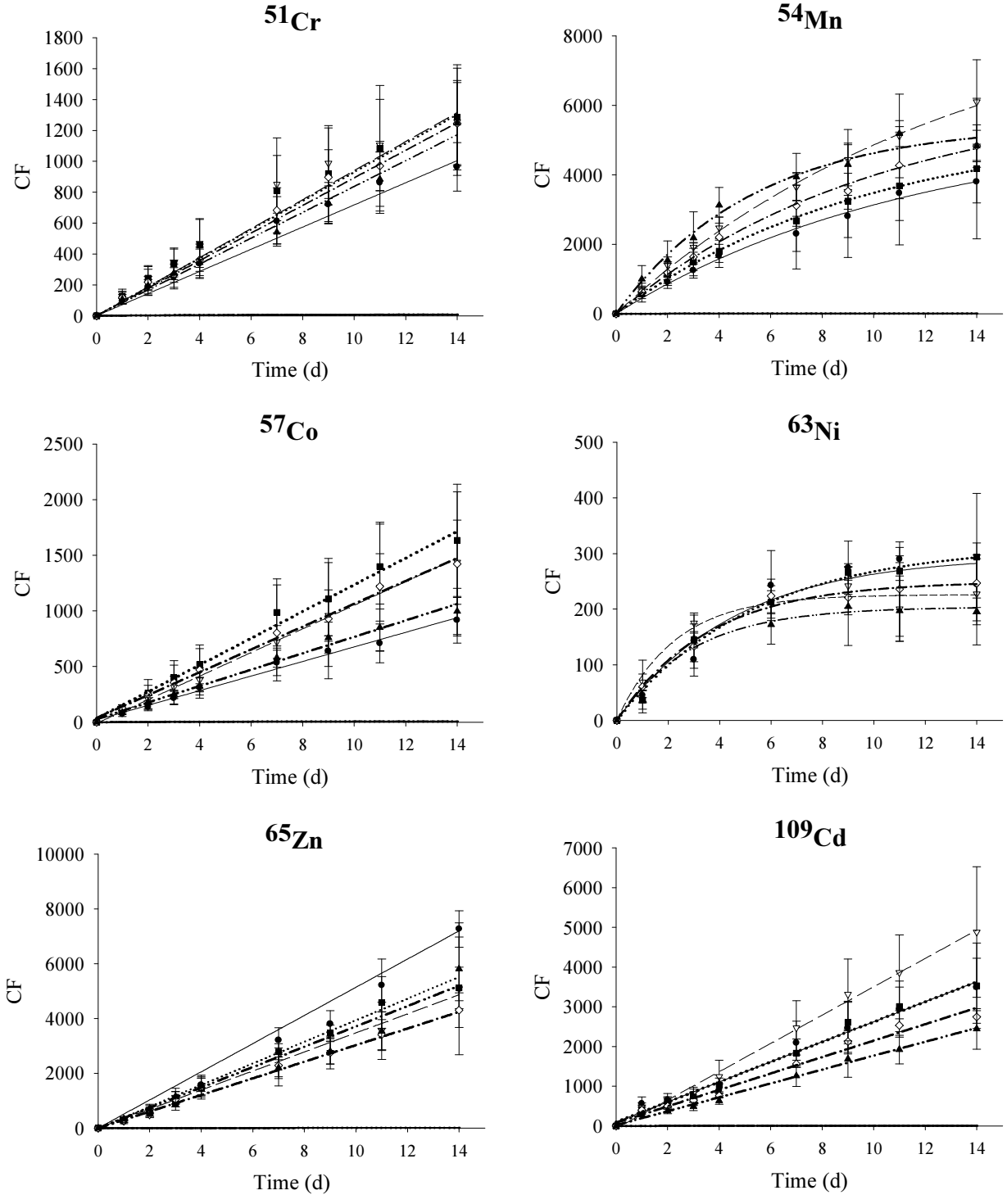

Figure 2. Influence of Cr, Mn, Co, Ni, Zn and Cd concentrations in dissolved exposure on subsequent whole body loss kinetics of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{63}Ni, ^{65}Zn and ^{109}Cd in *Lobophora variegata* (% Remaining activity, A(%), mean ± SD, n = 5, except for Ni, n = 3).

Parameters and statistics of the loss biokinetics and previous exposure concentrations are given in Table 1.

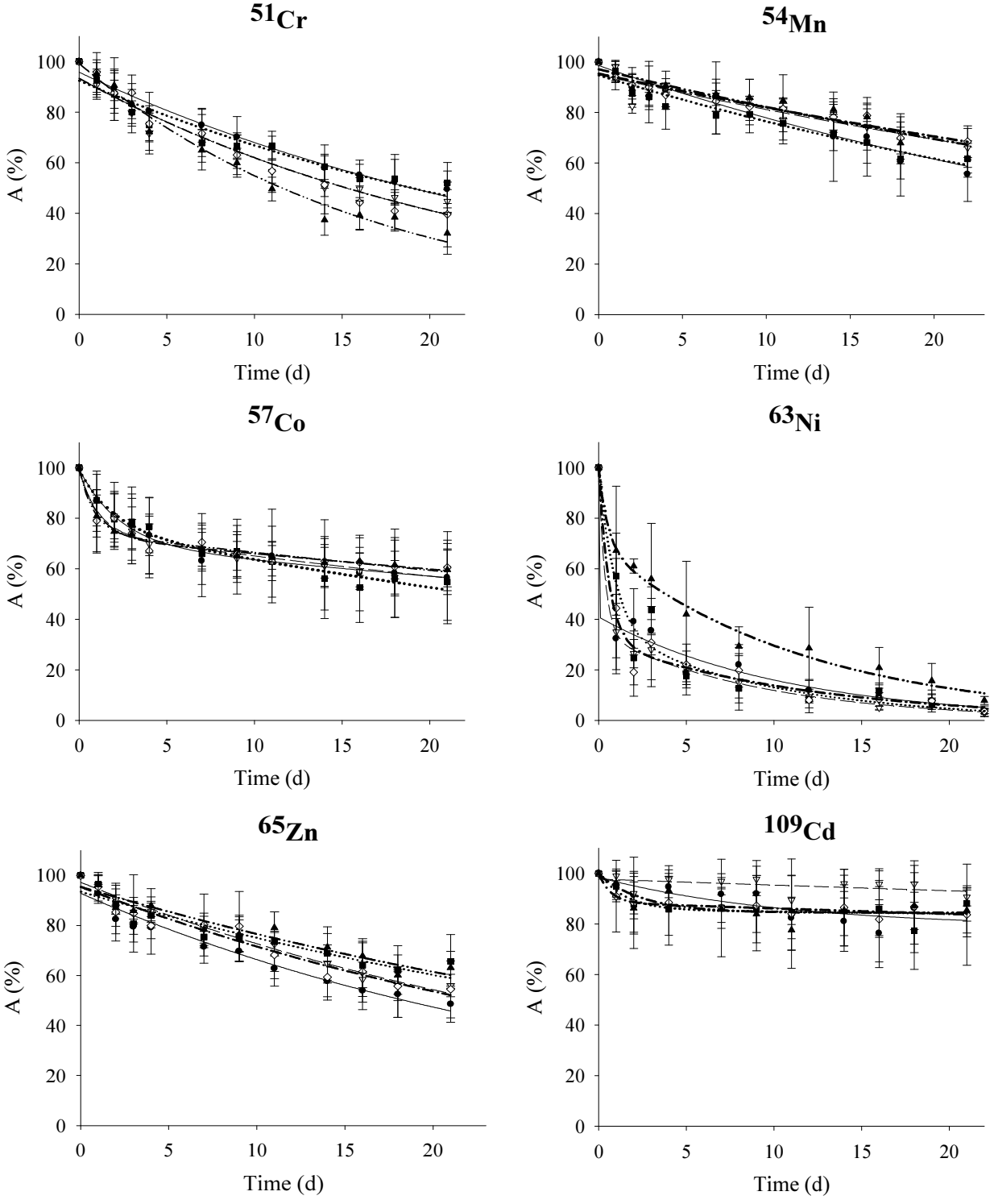

Table 1. Parameters and statistics of the equations best fitting the whole body uptake and loss kinetics of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{63}Ni, ^{65}Zn and ^{109}Cd in *Lobophora variegata*.

Uptake kinetics parameters for Linear model (L) and Exponential model (E): concentration factors at steady state (CF_{ss}) and uptake rate constant (k_u, d^{-1}). Loss kinetics parameters for Single-component exponential model (S) and Two-component loss exponential model (T): Remaining activities at time 0 (A_0,%), loss rate constant (k_e,d^{-1}) and biological half-life ($T_{b½}$,d). In the case of T model, only long-lived component parameters are shown (A_{0l}, k_{el} and $T_{b½\,l}$) ASE: asymptotic standard error; R^2: determination coefficient of the biokinetics. * $p < 0.0001$; ** $0.0001 < p < 0.001$; *** $0.001 < p < 0.05$

	Uptake phase							Depuration phase							
Isotopes	**Concentration (ng l^{-1})**	**Model**	**ku**	**ASE**	**CFss**	**ASE**	**R²**	**Model**	**A0**	**ASE**	**ke**	**ASE**	**T b½**	**ASE**	**R²**
^{51}Cr	0,1	**L**	76,1 *	2,4			**0,89**	**S**	95,78 *	1,7	0,03 *	0,002	20	1	**0,85**
	10,1	**L**	100,8 *	4,0			**0,83**	**S**	93,31 *	1,7	0,04 *	0,002	17	1	**0,86**
	50,1	**L**	99,2 *	4,7			**0,77**	**S**	92,41 *	1,7	0,03 *	0,002	21	1	**0,81**
	250,1	**L**	91,7 *	2,7			**0,91**	**S**	98,31 *	2,0	0,05 *	0,003	14	1	**0,87**
	1250,1	**L**	84,3 *	2,5			**0,91**	**S**	99,78 *	2,3	0,06 *	0,005	11	1	**0,56**
^{54}Mn	0	**E**	466,6 *	48	5440 *	1149	**0,9**	**S**	98,72 *	2,5	0,02 *	0,003	29	3	**0,6**
	10	**E**	688,7 *	72	9267 **	2621	**0,92**	**S**	94,98 *	1,5	0,02 *	0,002	44	4	**0,65**
	50	**E**	556,8 **	137	5428 ***	2021	**0,66**	**S**	94,89 *	1,5	0,02 *	0,002	32	2	**0,77**
	250	**E**	625,1 *	59	6377 *	976	**0,91**	**S**	95,48 *	1,1	0,02 *	0,001	45	3	**0,76**
	1250	**E**	1022,6 *	89	5465 *	332	**0,93**	**S**	97,18 *	1,5	0,02 *	0,002	41	4	**0,73**
^{57}Co	0,0	**L**	67,8 *	2,2			**0,89**	**T**	70,12 *	13,7	0,01	0,013	67	84	**0,47**
	8,0	**L**	105,0 *	3,6			**0,89**	**T**	74,16 *	7,3	0,01	0,007	53	28	**0,65**
	23,0	**L**	123,8 *	5,1			**0,83**	**T**	76,66 *	18,7	0,02	0,016	37	32	**0,61**
	248,0	**L**	106,7 *	7,2			**0,65**	**T**	72,74 *	5,6	0,01	0,006	71	41	**0,58**
	998,0	**L**	76,9 *	2,3			**0,9**	**T**	74,35 *	2,9	0,01 **	0,003	62	17	**0,77**
^{63}Ni	4	**E**	65,6 *	39	295 *	86	**0,77**	**T**	40,94 *	4,0	0,09 *	0,018	7	1	**0,92**
	19	**E**	101,3 **	82	226 *	47	**0,63**	**T**	33,88 *	5,0	0,10 **	0,025	7	2	**0,95**
	79	**E**	59,8 *	39	315 *	118	**0,8**	**T**	36,59 *	11,9	0,10 ***	0,041	7	3	**0,92**
	354	**E**	71,0 **	52	315 *	66	**0,72**	**T**	31,52 *	6,0	0,08 **	0,024	8	2	**0,91**
	1404	**E**	69,9 **	55	204 *	50	**0,69**	**T**	68,93 *	10,8	0,08 *	0,020	8	2	**0,84**
^{65}Zn	6	**L**	475,0 *	11			**0,94**	**S**	92,86 *	1,5	0,03 *	0,002	21	1	**0,86**
	76	**L**	337,2 *	18			**0,77**	**S**	97,20 *	1,3	0,03 *	0,002	24	1	**0,89**
	256,0	**L**	383,3 *	9,3			**0,94**	**S**	93,39 *	1,9	0,02 *	0,002	32	3	**0,66**
	706,0	**L**	313,3 *	7,2			**0,94**	**S**	95,37 *	2,5	0,03 *	0,003	24	3	**0,65**
	1756	**L**	361,0 *	12			**0,89**	**S**	95,40 *	1,4	0,02 *	0,002	31	2	**0,79**
^{109}Cd	0,0	**L**	263,0 *	9,1			**0,87**	**T**	94,91 *	3,1	0,008 ***	0,003	85	28	**0,31**
	2	**L**	350,0 *	14			**0,85**	**T**	96,47 *	2,0	0,002	0,002	447	472	**0,09**
	10,0	**L**	265,8 *	8,9			**0,88**	**T**	85,63 *	5,7	0,001	0,005	790	4159	**0,17**
	50,0	**L**	215,4 *	3,8			**0,96**	**T**	88,37 *	6,4	0,003	0,005	269	566	**0,07**
	250,0	**L**	177,2 *	5,3			**0,91**	**T**	86,99 *	4,1	0,005	0,004	151	116	**0,49**

When examining separately the estimated uptake kinetics parameters (k_u, CF_{14d}), no significant difference was observed among the different exposure concentrations in seawater for all metals, except for Mn. For this latter metal, although CF_{ss} was not significantly affected by increasing exposure concentration, k_u showed a slight but significant increase (slope of 0.37, $p < 0.02$) over the Mn concentration range considered (0 to 1250 ng l^{-1}). This relationship was no more significant (indicating independance of CF towards exposure concentration) when the range of Mn concentration was restricted up to 250 ng Mn l^{-1}.

At the end of the exposure period, seaweeds were placed for 21d (28d for Ni) in non contaminating condition to follow the loss kinetics of the radiotracers. Loss of ^{51}Cr, ^{54}Mn and ^{65}Zn was best described by a single exponential model (SEM) for all concentrations tested ($R^2 > 0.56$; Fig. 2, Table 1). For the three other radiotracers (^{57}Co, ^{63}Ni and ^{109}Cd), loss kinetics were best described by a two-component exponential model (TEM). Retention capacities of the alga varied according to the metal considered and ranked as follows: Cd > Cr = Zn = Mn = Co > Ni.

LMM analysis indicated that for all metals no significant difference was observed among loss kinetics determined over the concentration ranges examined (p_{SPSS} always > 0.05). Simple linear regressions between kinetic parameters (A_0, k_e $T_{b½}$ for SEM and A_{0l}, k_{el}, $T_{b½l}$ for TEM) and exposure concentrations indicated that none of these parameters did vary significantly with ambient metal levels over the whole concentration ranges tested (p_{slope} always > 0.05).

The long-lived component of ^{57}Co and ^{109}Cd loss kinetics represented the loss of the major proportion of the radioactivity (70 to 77% and 86 to 96%, respectively) and were characterized by long $T_{b½l}$, ranging from 37 d to the infinite (k_{el} not significantly different from 0). ^{63}Ni was the only metal for which the major fraction of incorporated activity (A_{0s} 31-69%) was rapidly lost ($T_{b½s}$ from 0.03 to 0.62 d; data not shown). In addition, $T_{b½}$ of the long lived component of the ^{63}Ni loss kinetics were short (6-8 d) compared to the $T_{b½}$ of the other metals.

IV. DISCUSSION

Among laboratory and field studies on Phaeophyceae spp. accumulation capacities (e.g. Bryan & Hummerstone 1973; Foster 1975; Burdon-Jones et al. 1982; Holmes et al. 1991; Miramand & Bentley 1992) and on their use in metal contamination biomonitoring programme (e.g. Phillips 1990; Amado Filho et al. 1999; Paez-Osuna et al. 2000), few studies

have actually investigated the relationships between concentration in brown algae and those in their ambient environment. To the best of our knowledge, only Bryan (1969) reported data on this crucial information in brown algae and observed that CF of Zn in *Laminaria digitata* decreased with increasing ambient concentrations.

The present study underscored the high capacity of *L. variegata* to concentrate dissolved metals and consequently, to allow for detection of low metal levels in seawater. Indeed, the alga accumulated metals up to concentration 120 to 7800 times (for Ni and Zn, respectively) higher than the concentration present in dissolved phase.

The present work also shown that, over the whole range of concentrations naturally encountered in the New Caledonia lagoon, the relative efficiency of metal uptake and retention was not affected by dissolved element concentrations for Cr, Co, Ni and Zn. Indeed, uptake and loss rate constants and CF of these metals were constant over the concentration range examined. In contrast, uptake kinetics of Cd and Mn were affected by the element concentration in seawater in *L. variegata*. However, in the case of Cd, this effect was not reflected when kinetics parameters were tested individually. On the other hand, although the CF_{ss} of Mn in alga showed no significant difference over the tested range of concentrations, its uptake rate constant (k_u) was affected by the highest Mn exposure concentration (1250 ng Mn l^{-1}). This effect would however mainly affect the initial phase of the uptake behaviour of the algae, since estimated CF_{ss} at 1250 ng Mn l^{-1} was found to be not significantly from those assessed for the lower concentration tested.

These observations indicate that the alga could be used as a Mn bioindicator, but over a restricted range of concentration (0 to 250 ng Mn l^{-1}) since k_u and k_e were only affected by Mn concentrations > than 250 ng l^{-1}. Nevertheless, as CF_{ss} has been shown not being affected by exposure concentrations, if algae remain in the field for more than 1 month (time needed to reach the CF_{ss}) and that no major variation in contamination levels occur during that period (which could affect k_u and/or k_e), *Lobophora variegata* could be used also to characterize location where higher Mn concentration (> 1250 ng Mn l^{-1}) do occur.

With the exception of Ni, all elements were efficiently retained by the alga ($T_{b½}$ higher than a week), indicating that it would be able to preserve information regarding contamination events over a relatively long period of time. Furthermore, retention of the 6 tested metals in *L. variegata* was similar whatever the previous exposure dissolved concentrations. A similar observation had been reported from temperate brown algae (Bryan 1969).

Several studies have investigated metal accumulation mechanisms (adsorption and absorption) in brown algae species (e.g. Phillips 1990). It was demonstrated that metals in solution bind to the cell walls of the macroalgae through a process approximating ion exchange and various authors have noted the high affinity of trace elements for polysaccharides such as alginates, which are present in the cell walls (e.g. Ragan & Jensen 1979; Veroy et al. 1980; Davis et al. 2003). Besides these adsorption (or biosorption) related processes, mechanisms of absorption were pointed out in Phaeophycee such as sequestration or chelation, where metals cross the cell and bind strongly to intracellular macromolecules such as polyphenols, phytochelatins (PC) and metallothioneins (MT) (e.g. Ragan et al. 1979; Phillips 1994b; Morris et al. 1999; Cobbett & Goldsbrough 2002).

From our results, it is not obvious to point out the involvement of one or several of these mechanisms in metal accumulation in *L. variegata*. Nevertheless, Ni has a contrasting behaviour in the alga compared to the other metals investigated. Indeed, Ni CFs were drastically lower and more rapidly reached a steady state equilibrium. Moreover, loss phase revealed the lower retention of Ni by *L. variegata* compared to others metals. All these characteristics strongly suggest that *L. variegata* has a low affinity for Ni and that Ni forms weak binding with algal intra-cellular components. Therefore, the major mechanism governing Ni bioconcentration in the alga would be most probably related to adsorption processes. Our assumption is reinforced by the work of Holan & Volesky (1993) which focuses on the use of algal biomass to immobilize Ni. These authors showed that the algae belonging to Phaephyceae Class provide the best biosorption natural material.

In contrast to Ni, all other metals showed high bioconcentration and efficient retention in the alga. It can thus be suggested that bioconcentration of these elements results from a combination of adsorption and absorption processes. Targett et al. (1992) indicated that as all brown algae, *L. variegata* has a high polyphenolic content (8.33 % to 13.39 % of dry weight) that could be involved in metal sequestration.

In conclusion, our work showed that *L. variegata* is characterised by a high capacity of bioconcentrating and depurating metals (except Ni). The present findings are of prime importance, since they demonstrate that metal concentrations measured in the macroalgae actually reflect those occurring in their environment. This seaweed species could be thus considered as a valuable bioindicator to monitor dissolved metal contamination in New Caledonia. Considering that *L. variegata* is a common species in tropical areas (Targett et al. 1992a), its used could be extended geographically. In particular, this species could be

considered to develop metal contamination metal contamination monitoring programme at a large scale in tropical zone.

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CHAPITRE 7

Influence de la nourriture sur l'assimilation des métaux dans des bivalves tropicaux : Aspects qualitatifs et quantitatifs

Le clam *Gafrarium tumidum* et l'huître *Isognomon isognomon* ont été exposés aux ^{54}Mn, ^{57}Co et ^{65}Zn par l'intermédiaire de la nourriture sous différentes conditions d'alimentation (souches de phytoplancton, densité cellulaire, et concentrations métalliques associées aux cellules phytoplanctoniques). Les résultats indiquent que la concentration en Co associée au phytoplancton n'a pas d'influence sur l'efficacité d'assimilation (EA) et sur le temps de rétention du Co dans les deux bivalves. Cependant, lorsque les bivalves sont nourris avec *Heterocapsa triquetra*, *Emiliania huxleyi* ou *Isochrysis galbana*, l'EA du Co, Mn et Zn est fortement influencée par le type de souche phytoplanctonique et par le métal considérés. Les métaux ingérés avec *I. galbana* montrent généralement une EA supérieure, sauf pour le Mn dans les clams, pour lesquels l'EA la plus élevées est observée pour *H. triquetra.* L'influence de la quantité de nourriture a été étudiée en exposant les bivalves à différentes densités cellulaires d'*I. galbana* ($5\ 10^3$, 10^4 et $5\ 10^4$ cell ml^{-1}). Comme pour la qualité de la nourriture, la quantité de nourriture influence l'EA du Co, Mn et Zn dans les bivalves, la plus forte AE étant observée quand les bivalves sont nourris avec la plus faible densité cellulaire. Par conséquent, nos résultats suggèrent que les deux bivalves sont capables d'ajuster leurs stratégies alimentaires selon les conditions d'alimentation prévalent dans l'environnement.

Influence of food on the assimilation of selected metals in tropical bivalves: qualitative and quantitative aspects

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ABSTRACT. The clam *Gafrarium tumidum* and the oyster *Isognomon isognomon* were exposed to ^{54}Mn, ^{57}Co and ^{65}Zn via their food under different feeding conditions (phytoplankton strain, cell density, and cell-associated metal concentration). Results indicated that the phytoplankton-associated Co concentration had no influence on the assimilation efficiency (AE) and on the retention time of the metal in both bivalves. In contrast, when bivalves were fed *Heterocapsa triquetra, Emiliania huxleyi* and *Isochrysis galbana*, AE of Mn, Co and Zn was strongly influenced by the phytoplankton strain and by the metal considered. Metal ingested with *I. galbana* displayed generally the highest AE, except for Mn in clams for which the highest AE was observed for *H. triquetra.* Influence of food quantity was investigated by exposing bivalves to different cell densities of *I. galbana* (5 10^3, 10^4 or 5 10^4 cell ml^{-1}). As for food quality, food quantity was found to influence Mn, Co and Zn AEs, the highest AE being observed when bivalves fed the lowest cell density. Overall, results suggest that the two bivalve species are able to adjust their feeding strategies according to the food conditions prevailing in their environment.

Keywords : *Gafrarium tumidum, Isognomon isognomon*, New Caledonia, Radiotracer, Feeding

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I. INTRODUCTION

Changes in coastal ecosystem functioning due to anthropogenic metal inputs is a worldwide issue especially as metals are not biodegradable, are present under various physicochemical forms and enter biogeochemical cycles (Tessier & Turner 1995). In the coral reef lagoon of New-caledonia, metal contamination is an even more specific and acute problem due to the confrontation between the extreme biodiversity of the system and the increasing environmental pressure imposed by urban development and intensive mining activities. Ni and Co extraction processes based on acidic extraction (lixiviation) have been recently developed and are expected to be applied at industrial scale in the very near future (Goro-Nickel 2001). Such processes will provide new potential to exploit ores with Ni contents lower than those currently utilized (Mihaylov et al. 2000; Dalvi et al. 2004). Acid extraction is an unselective process that will result in the additional discharge of unretained by-product metals (Cr, Mn, Zn, etc.) (Goro-Nickel 2001; Baroudi et al. 2003).

Although long lasting contamination exists in New Caledonia (Laganier 1991; Ambatsian et al. 1997), few data on contamination levels and possible local marine ecosystem impairments are available(e.g. Monniot et al. 1994). Therefore, programmes for biomonitoring mining contamination in the New Caledonian lagoon are strongly needed and should largely rely on the use of bioindicator species, as in temperate areas (e.g. Mussel Watch, Goldberg et al. 1978). In this context, the clam *Gafrarium tumidum* and the oyster *Isognomon isognomon* have been identified as promising bioindicator candidates (Breau 2003). These species were further shown to efficiently accumulate and retain metals when exposed via seawater, food or sediment and to allow the discrimination of different geographical areas according to the ambient contamination levels (Hédouin et al. submitted-a; Hédouin et al. submitted-d; Hédouin et al. to be submitted-a, to be submitted-b). Food intake was also identified as an important pathway for metal bioaccumulation in these filter-feeding organisms (Wang et al. 1996; Hédouin et al. to be submitted-a, to be submitted-b).

Bioaccumulation of metals ingested with food is strongly influenced by assimilation efficiency (AE) (Thomann et al. 1995; Wang & Fisher 1999b). This has been well documented for example in mussels (e.g. Wang & Fisher 1997a), polychaetes (e.g. Wang et al. 1999) and copepods (e.g. Wang & Fisher 1998). Furthermore, feeding processes in marine organisms are flexible according to the changes in environmental conditions such as food

quantity and quality (Widdows & Donkin 1992; Navarro & Iglesias 1993). For example, metal assimilation in the mussel *Mytilus edulis* was inversely related to food quantity (Borchardt 1983). Bivalves are also able to select ingested particles (Iglesias et al. 1996) which results in a preferential ingestion of nutritionally-rich particles that may affect metal influx from food (Bayne 1993; Wang & Fisher 1997a).

The objective of this study was to investigate the influence of food quality and quantity on the assimilation efficiency of three metals (Co, Mn and Zn) in the clam *G. tumidum* and the oyster *I. isognomon*. The variations in feeding conditions studied were 1) the phytoplankton strains used as food, 2) the phytoplankton density and 3) the metal concentration associated with phytoplankton. Radiotracer techniques were used to enhance the detection sensitivity of metals and to allow for measuring metal flux at realistic contaminant concentrations.

II. MATERIALS AND METHODS

II.1. COLLECTION AND ACCLIMATION

The organisms (n = 100 per species) were hand-picked in Dumbea Bay (clams *Gafrarium tumidum*) or collected by SCUBA diving in Maa Beach (oysters *Isognomon isognomon*), Nouméa, New Caledonia in October 2003. Body size affects bioaccumulation of metals in marine organisms (Boyden 1974) and, according to previous studies (Metian 2003; Hédouin et al. in press), only individuals with a shell width longer than 35 mm (*G. tumidum*) or a shell length longer than 70 mm (*I. isognomon*) were collected. Oysters and clams were shipped to IAEA-MEL premises in Monaco, where they were acclimated for 2 months to laboratory conditions (open circuit aquarium; water renewal rate: 10 % hr^{-1}; salinity: 36 p.s.u.; temperature T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) simulating the conditions prevailing in the New Caledonian lagoon. During acclimation, bivalves were fed phytoplankton (*Isochrysis galbana*); recorded mortality was lower than 5%.

II.2. RADIOTRACERS AND COUNTING

Investigated elements (Co, Mn, and Zn) were introduced into the experimental microcosm as radiotracers of high specific activity purchased from Amersham, UK (^{57}Co in 0.1M HCl, $T_{½}$ = 271.8 d) and Isotope Product Lab., USA (^{65}Zn in 0.5M HCl; $T_{½}$ = 243.9 d; ^{54}Mn in 0.1M HCl, $T_{½}$ = 312.2 d). Radioactivity was measured using a high-resolution γ-spectrometer system composed of 3 Germanium N or P type detectors (EGNC 33-195-R, Intertechnique)

connected to a multichannel analyser (Intergamma, Intertechnique). The radioactivity of the samples was determined by comparison with standards of known activities and of appropriate geometry. Measurements were corrected for counting efficiency, background and radioactive decay. Counting times were adapted to obtain counting rates with propagated errors less than 5%.

II.3. EXPERIMENTAL PROCEDURE

II.3.1. Testing the influence of Co concentration in food

Cells of *Isochrysis galbana* from an axenic stock culture were resuspended into 4 erlenmeyer flasks (light/dark cycle: 12 hrs/12 hrs at 25°C). Each flask contained 500 ml sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si. Flasks were spiked with 4 increasing Co concentrations (0, 5, 50, 500 ng l^{-1}) and phytoplankton was allowed to grow under these conditions for 6 d. Added Co concentrations were realized using increasing amount of $Co(NO_3)_2$ (synthesis quality, Merck) and a fixed activity of the corresponding radiotracer ^{57}Co (2.5 kBq l^{-1}, corresponding to 0.13 ng Co l^{-1}). The range of concentrations selected covers those encountered in the New Caledonia lagoon waters (Goro-Nickel 2004). After 6 d of incubation, cell density increased from 10^3 to 1.5 10^6 cell ml^{-1}. The cells were gently filtered (1µm-mesh size, Nuclepore® Polycarbonate filters) and resuspended in clean seawater. The radioactivity of the radiolabelled *I. galbana* in each flask was γ-counted before and after the filtration. The radioactivity of algal cells used in feeding experiments was not significantly different among the different flasks, with an average activity of 0.49 ± 0.14 µBq $cell^{-1}$.

For each added Co concentration, 4 groups of 9 oysters (shell length from 71 to 94 mm) and 4 groups of 9 clams (shell width from 35 to 40 mm) were dispatched in 4 aquaria containing 16 l of filtered natural seawater (close circuit aquaria constantly aerated, salinity: 36 p.s.u.; T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) and acclimated for one week to these conditions (daily water renewal). Bivalves from each aquarium were then allowed to feed for 2 hours on one out of the 4 batches of previously radiolabelled *I. galbana* (10^4 cell ml^{-1}).

Control empty shells were placed in each aquarium to check for any direct uptake of radiotracers from seawater due to possible leaching of radiotracers from phytoplankton cells

during the 2-hr feeding period. These control shells were radioanalysed at regular intervals of time.

After the feeding period, all organisms were γ-counted and open circuit conditions were restored (water renewal rate: 10 % hr^{-1}; salinity: 36 p.s.u.; T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). All individuals were then γ-counted at different time intervals during 25 d to determine the whole-body loss kinetics of the radiotracers ingested with food and associated kinetic parameters.

II.3.2. Testing the influence of phytoplankton strains

Experimental approaches conducted on *I. galbana* were applied to *Emiliania huxleyi* and *Heterocapsa triquetra* so as to asses the possible influence of phytoplankton strains. Cells from axenic stock cultures were resuspended in two different erlenmeyer flasks (10^3 cell ml^{-1}), containing 4500 ml sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si for *H. triquetra* and enriched with f/50 for *E. huxleyi.* The two cultures were spiked with 5 kBq l^{-1} of ^{54}Mn, ^{57}Co and ^{65}Zn. The cultures were then incubated for 6 d at 25°C (light/dark cycle: 12 hrs/12 hrs). After incubation, the cell density had increased from 10^3 to 7 10^5 cell ml^{-1} for *E. huxleyi* and to 1.6 10^5 cell ml^{-1} for *H. triquetra*. The cells were then gently filtered, resuspended in clean seawater and γ-counted as described above. The radioactivity of algal cells used in the feeding experiments was 0.26 ± 0.18 µBq $cell^{-1}$ for *E. huxleyi* and 0.96 ± 0.11 µBq $cell^{-1}$ for *H. triquetra* for ^{54}Mn, 2.1 ± 0.8 and 20.8 ± 12.1 µBq $cell^{-1}$ for ^{57}Co and 3.2 ± 1.3 and 3.3 ± 0.1 µBq $cell^{-1}$ for ^{65}Zn.

For each phytoplankton strain tested, 2 groups of 9 oysters (shell length from 73 to 90 mm) and 2 groups of 9 clams (shell width from 35 to 44 mm) were dispatched in 2 aquaria containing 16 l of filtered natural seawater (close circuit aquaria constantly aerated, salinity: 36 p.s.u.; T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs), acclimated for 1 week (daily seawater renewal) and then fed radiolabelled *E. huxleyi* and *H. triquetra* (10^4 cell ml^{-1}) for 2 hours. Controls for assessing possible direct uptake through radiotracer leaching from algal cells and determination of whole-body loss kinetics of radiotracer ingested with the food were performed as described in section II.3.1.

II.3.3. Testing the influence of cellular density

Cells of *I. galbana* from an axenic stock culture were resuspended in an erlenmeyer flask containing 4.5 l sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si.

The culture was then spiked with 5 kBq l^{-1} of ^{54}Mn, ^{57}Co and ^{65}Zn and incubated for 6 d at 25°C (light/dark cycle: 12 hrs/12 hrs). After incubation, the cell density had increased from 10^3 to 1.4 10^6 cell ml^{-1}. Three sub-samples of 58, 115 and 580 ml of the culture were then gently filtered and resuspended in clean seawater. These 3 batches were prepared to obtain final cell density of 5 10^3, 10^4 and 5 10^4 cell ml^{-1} in the 16-l exposure aquaria. The radioactivity of the radiolabelled *I. galbana* was measured before and after the cellular filtration. The radioactivity of algal cells ranged from 1.11 to 1.80 µBq $cell^{-1}$ for ^{54}Mn, 0.83 to 1.37 µBq $cell^{-1}$ for ^{57}Co, 2.69 to 4.38 µBq $cell^{-1}$ for ^{65}Zn (the highest radioactivities were counted in the lowest cellular density).

Three groups of 9 oysters (shell length from 71 to 92 mm) and 3 groups of 9 clams (shell width from 36 to 45 mm) were dispatched in 3 aquaria containing 16 l of filtered natural seawater (close circuit aquaria constantly aerated, salinity: 36 p.s.u.; T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs), and acclimated for one week (daily seawater renewal). Each group of clams and oysters was then fed for 2hrs one of radiolabelled the *I. galbana* batches (5 10^3, 10^4 or 5 10^4 cell ml^{-1}).

Controls for assessing possible direct uptake through radiotracer leaching from alga cells and determination of whole-body loss kinetics of radiotracer ingested with the food were performed as described in section II.3.1.

II.4. DATA ANALYSES

Losses of radiotracers were expressed as the percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in the organisms just after the feeding period). The loss kinetics were best fitted by a double-component exponential equation:

$$A_t = A_{0s}\, e^{-k_{es}\, t} + A_{0l}\, e^{-k_{el}\, t}$$

where k_e is the depuration rate constant (d^{-1}); A_t, A_0 are the remaining activities (%) at time t (d) and 0, respectively; ‘s’ and ‘l’ are the subscripts for the short-lived and long-lived components. The short-lived component represents the loss kinetics of the radiotracer fraction that remains associated with the faeces and is rapidly eliminated with them, while the long-lived component describes the loss kinetics of the radiotracer fraction that is actually absorbed by the organism and slowly eliminated (Whicker & Schultz 1982). The long-lived component allows assessing the assimilation efficiency (AE) of the radiotracer ingested with food (AE =

A_{0l}). Also, for each exponential component (s and l), a biological half-life can be calculated ($T_{b½s}$ and $T_{b½l}$, respectively) from the corresponding depuration rate constant (k_{es} and k_{el}) according to the relation $T_{b½} = \ln 2/k_e$.

Constants of the models and their statistics were estimated by iterative adjustments of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica® 5.2.1 software. Best fitting regression models were selected according to highest determination coefficient and examination of residuals. Differences among the estimated kinetic parameters of the different feeding conditions were tested using mean comparisons tests. Possible trends linking metal concentrations to cell densities were assessed using simple linear regression techniques (Zar 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

Loss kinetics of radiotracers were followed in the organisms which actually ingested enough food to display sufficient radioactivity to be accurately counted. Most oysters met this requirement; however some clams displaying very low activities were discarded. No activity was detected on control shells, indicating that no detectable recycling of phytoplankton-associated tracers occurred in the experimental microcosms.

III.1. EFFECT OF CO CONCENTRATION IN PHYTOPLANKTON

Whole-body loss kinetics of ^{57}Co in oysters were best described by a double-exponential model (R^2: 0.86-0.90) for all Co concentrations tested (Table 1, Fig. 1). The major fraction (80-85 %) of the total radioactivity in oysters was rapidly lost ($T_{b½s} < 1$d). The long-lived component accounted for 15-20 % of the ^{57}Co ingested with food and presented a biological half life ($T_{b½l}$) ranging from 13 to 25 d.

Whole-body loss kinetics of ^{57}Co in clams were best described by a double-exponential model (R^2: 0.27-0.64) for all Co concentrations tested (Table 1, Fig. 1). The estimated AE of ^{57}Co ingested with food ranged from 76 to 84 %, and this fraction was retained with a $T_{b½}$ ranging from 36 to 39 d.

Statistical analyses indicated that, for both bivalves, no significant difference was found among the estimated kinetic parameters (A_{0s}, k_{es}, A_{0l}, k_{el}) determined for the 4 different food-associated Co concentrations.

Table 1. Assimilation efficiency (AE, %), loss rate constant (k_{el}, d^{-1}) and biological half-life ($T_{b½l}$, d) of ^{57}Co in whole-body bivalves fed *Isochrysis galbana* previously exposed to four increasing Co concentrations (n = 9 *Isognomon isognomon* per concentration tested, n = 5 *Gafrarium tumidum* for C_0 and C_3 and n = 8 for C_1 and C_2).

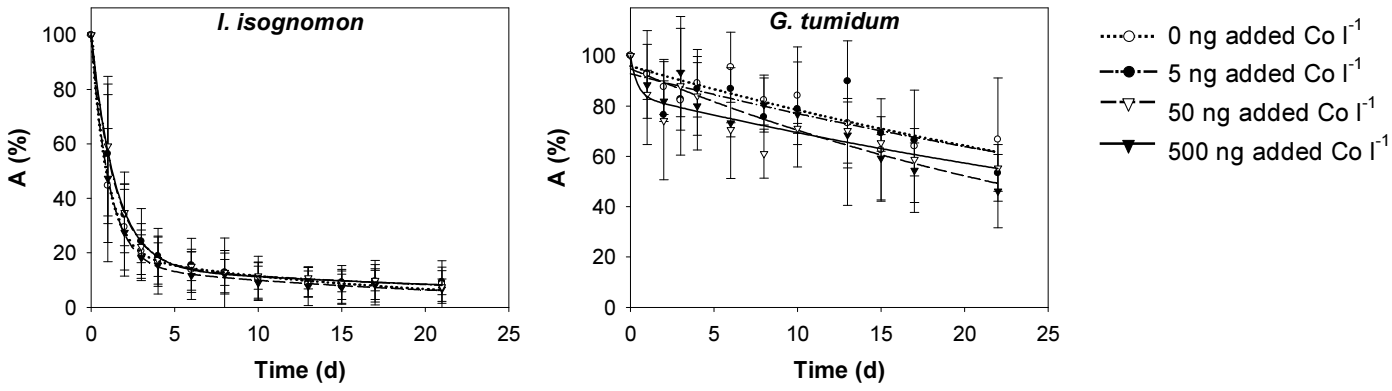
ASE: asymptotic standard error; R^2: determination coefficient.

Species	Co concentration added	AE ± ASE	k_{el} ± ASE	$T_{b½l}$ ± ASE	R^2
I. isognomon	0 ng l^{-1}	15.8 ± 7.0 [a]	0.032 ± 0.036*	22 ± 24*	0.87
	5 ng l^{-1}	19.6 ± 5.4 [c]	0.054 ± 0.028*	13 ± 7*	0.88
	50 ng l^{-1}	16.6 ± 6.2 [b]	0.050 ± 0.036*	14 ± 10*	0.86
	500 ng l^{-1}	14.7 ± 6.0 [a]	0.027 ± 0.033*	25 ± 30*	0.90
G. tumidum	0 ng l^{-1}	77.2 ± 3.9 [d]	0.018 ± 0.006 [b]	37. ± 11 [b]	0.64
	5 ng l^{-1}	77.4 ± 3.9 [d]	0.019 ± 0.006 [c]	36 ± 10 [c]	0.27
	50 ng l^{-1}	75.7 ± 3.9 [d]	0.018 ± 0.006 [b]	39 ± 12 [b]	0.51
	500 ng l^{-1}	84.1 ± 5.9 [d]	0.019 ± 0.007 [a]	36 ± 13 [a]	0.51

Significance of the estimated parameters: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, [d] $p < 0.0001$, * $p > 0.05$

Figure 1. Influence of stable Co concentrations in phytoplankton (*Isochrysis galbana,* 10^4 cell ml^{-1}) on whole-body loss kinetics of ^{57}Co in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* previously fed for 2 hrs radiolabelled phytoplankton.

A: mean remaining activity (%) ± SD.



III.2. EFFECT OF CELLULAR DENSITY

Whole-body loss kinetics of radiotracers in oysters fed *I. galbana* at 10^4 and 5 10^4 cell ml^{-1} were best described by a double-exponential model (R^2: 0.23-0.63 and 0.38-0.60, respectively) (Table 2, Fig. 2). Biological half-lives ($T_{b½}$) and AE varied as a function of cell density and the metal considered. For Co, statistical analysis indicated that $T_{b½}$ and AE for both cellular densities were not significantly different. In contrast, significant differences were found for Mn and Zn, higher AE corresponding to lower cell density ($p = 0.001$ and 0.0003, respectively).

In clams fed *I. galbana,* loss kinetics of the three radiotracers were best described by a double-exponential model (R^2: 0.33-0.65 at 5 10^3 cell ml^{-1} and 0.47-0.98 at 10^4 cell ml^{-1}) (Table 2, Fig. 3). Statistical analysis indicated that $T_{b½}$ was not significantly different between both cellular densities for all three radiotracers. However, when fed the lowest cell density, clams incorporated Co, Mn and Zn with significantly higher AE ($p = 0.003$, 0.047 and 0.0003, respectively).

Figure 2. Influence of phytoplankton cell density on whole-body loss kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn in the oyster *Isognomon isognomon* previously fed for 2 hrs radiolabelled *Isochrysis galbana* at 10^4 cell ml^{-1} and 5 10^4 cell ml^{-1}.

A: mean remaining activity (%) ± SD

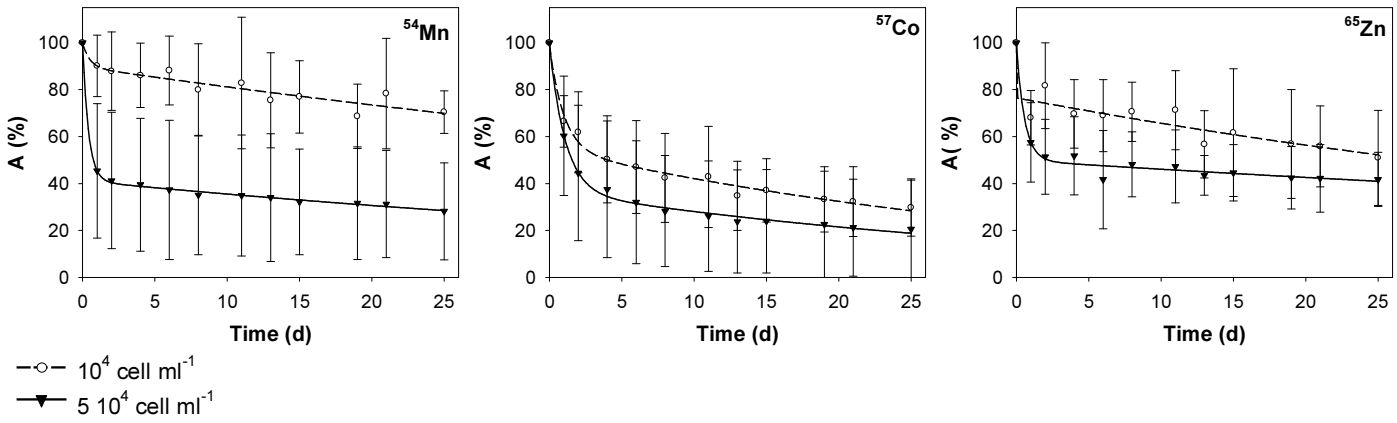


Table 2. Assimilation efficiency (AE, %), loss rate constant (k_{el}, d^{-1}) and biological half-life ($T_{b½l}$, d) of ^{54}Mn, ^{57}Co and ^{65}Zn in whole-body bivalves fed *Isochrysis galbana* at different cell densities (n = 9 *Isognomon isognomon*; n = 6 *Gafrarium tumidum* for 5 10^3 and n = 8 for 10^4 cell ml^{-1}).

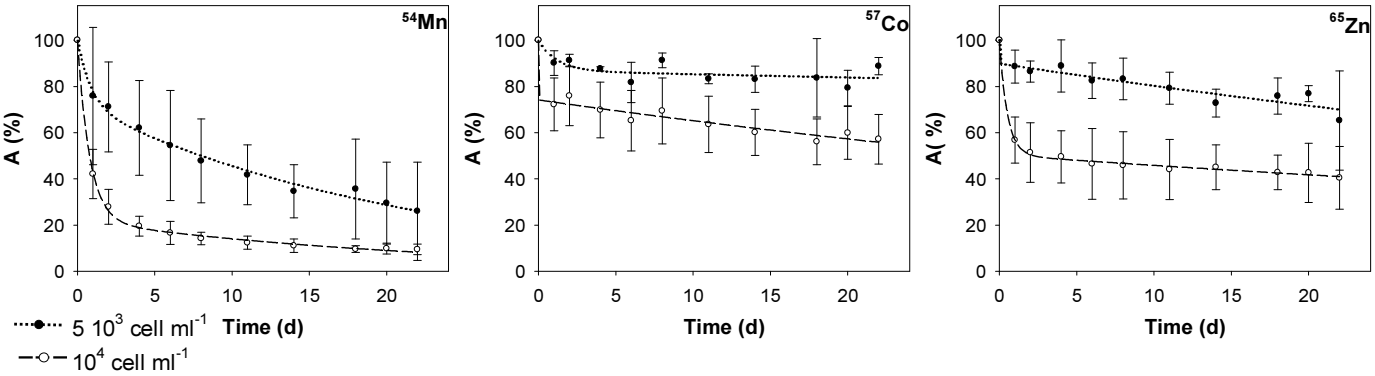
ASE: asymptotic standard error; R^2: determination coefficient.

Species	Cell density	Isotope	AE ± ASE	$k_{e\,l}$ ± ASE	$T_{b½l}$ ± ASE	R^2
I. isognomon	**10^4 cell ml^{-1}**	^{54}Mn	90 ± 5.6 ^{d}	0.010 ± 0.005 ^{a}	70 ± 32	0.23
		^{57}Co	55 ± 7.1 ^{d}	0.026 ± 0.01 ^{b}	26 ± 10	0.63
		^{65}Zn	76 ± 4.1 ^{d}	0.015 ± 0.004 ^{b}	45 ± 13	0.36
	5 10^4 cell ml^{-1}	^{54}Mn	41 ± 7.8 ^{d}	0.015 ± 0.014*	47 ± 46	0.38
		^{57}Co	367 ± 21.2*	0.027 ± 0.043*	26 ± 42	0.51
		^{65}Zn	52 ± 3.5 ^{d}	0.010 ± 0.005^{a}	70 ± 34	0.60
G. tumidum	**5 10^3 cell ml^{-1}**	^{54}Mn	72 ± 17.9 ^{c}	0.046 ± 0.021^{a}	15 ± 7	0.65
		^{57}Co	87 ± 6.1^{d}	0.002 ± 0.005*	416 ± 135	0.33
		^{65}Zn	90 ± 3.0 ^{d}	0.011 ± 0.003 ^{c}	61 ± 16	0.52
	10^4 cell ml^{-1}	^{54}Mn	22 ± 3.7 ^{d}	0.044 ± 0.015 ^{b}	16 ± 5	0.98
		^{57}Co	73 ± 2.7 ^{d}	0.010 ± 0.003 ^{b}	68 ± 21	0.47
		^{65}Zn	51 ± 3.8 ^{d}	0.013 ± 0.006 ^{a}	55 ± 25	0.68

Significance of the estimated parameters: ^{a} $p < 0.05$, ^{b} $p< 0.01$, ^{c} $p< 0.001$, ^{d} $p< 0.0001$, * $p > 0.05$

Figure 3. Influence of phytoplankton cell density on whole-body loss kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn in the clam *Gafrarium tumidum* previously fed for 2 hrs radiolabelled phytoplankton *Isochrysis galbana* at 5 10^3 cell ml^{-1} and 10^4 cell ml^{-1}.

A: mean remaining activity (%) ± SD.



III.3. EFFECT OF PHYTOPLANKTON STRAINS

Loss kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn in oysters fed radiolabelled *I. galbana* (ISO), *H. triquetra* (HET) and *E. huxleyi* (EMI) at 10^4 cell ml^{-1} were best described by a double-exponential model (R^2: 0.23- 0.63 for ISO, 0.57-0.92 for HET and 0.11-0.83 for EMI) (Tables 2 and 3, Fig. 4). No significant difference was found among $T_{b½}$ for all radiotracers and all phytoplankton strains tested, nor among AEs for Co and Mn in oysters fed HET and EMI, and for Zn in oysters fed EMI and ISO. In contrast, significant differences ($p < 0.02$) among AEs have been observed for Co, Mn and Zn (Zn: EMI = ISO > HET, $p < 0.02$; Co and Mn: ISO > HET = EMI, $p < 0.004$).

In clams, whole-body loss kinetics of the radiotracers ingested with *I. galbana*, *H. triquetra* or *E. huxleyi* (10^4 cell ml^{-1}) were best described by a double-exponential model (R^2: 0.47-0.98 for ISO, 0.61-0.89 for HET and 0.47-0.93 for EMI) (Tables 2 and 3, Fig. 5). $T_{b½}$ of ingested Mn was significantly longer for HET than for EMI or ISO ($p = 0.03$ and 0.004, respectively). Significant differences were also observed among AEs calculated for Co, Mn and Zn ingested with the three phytoplankton strains (Mn: HET = EMI > ISO, $p < 0.03$; Co: EMI = ISO > HET, $p < 0.0004$; and Zn: ISO > HET, $p = 0.04$).

Table 3. Assimilation efficiency (AE, %), loss rate constant (k_{el}, d^{-1}) and biological half-life ($T_{b½l}$, d) of ^{54}Mn, ^{57}Co and ^{65}Zn in whole-body bivalves fed radiolabelled *Heterocapsa triquetra* and *Emiliania huxleyi* (n = 8 *Isognomon isognomon,* n = 7 *Gafrarium tumidum* per phytoplankton strains tested).

ASE: asymptotic standard error; R^2: determination coefficient.

Species	Phytoplankton strain	Isotope	AE ± ASE	k_{el} ± ASE	$T_{b½l}$ ± SD	R^2
I. isognomon	***H. triquetra***	^{54}Mn	20 ± 2.6 ^{d}	0.025 ± 0.012 ^{a}	28 ± 13 ^{a}	0.92
		^{57}Co	21 ± 4.1 ^{d}	0.050 ± 0.020 ^{a}	14 ± 6 ^{a}	0.88
		^{65}Zn	51 ± 3.2 ^{d}	0.006 ± 0.005*	123 ± 109*	0.57
	E. huxleyi	^{54}Mn	34 ± 6.5 ^{d}	0.028 ± 0.015*	24 ± 13*	0.74
		^{57}Co	22 ± 5.6 ^{c}	0.039 ± 0.021*	18 ± 10*	0.83
		^{65}Zn	70 ± 6.5 ^{d}	0.0002 ± 0.007*	2783*	0.11
G. tumidum	***H. triquetra***	^{54}Mn	56 ± 23 ^{a}	0.021 ± 0.026*	34 ± 10*	0.61
		^{57}Co	41 ± 3.1 ^{d}	0.014 ± 0.006 ^{a}	49 ± 9 ^{a}	0.89
		^{65}Zn	33 ± 7.3 ^{d}	0.005 ± 0.016*	143 ± 474*	0.71
	E. huxleyi	^{54}Mn	39 ± 4.3 ^{d}	0.051 ± 0.011 ^{d}	14 ± 3 ^{d}	0.92
		^{57}Co	80 ± 3.5 ^{d}	0.010 ± 0.004 ^{a}	70 ± 26 ^{a}	0.47
		^{65}Zn	42 ± 2.3 ^{d}	0.014 ± 0.004 ^{b}	48 ± 14 ^{b}	0.93

Significance of the estimated parameters: ^{a} $p < 0.05$, ^{b} $p< 0.01$, ^{c} $p< 0.001$, ^{d} $p< 0.0001$, * $p > 0.05$

Figure 4. Influence of phytoplankton strains (*Heterocapsa triquetra*, HET, *Emiliania huxleyi*, EMI and *Isochrysis galbana*, ISO; 10^4 cell ml^{-1}) on whole-body loss kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn in the oyster *Isognomon isognomon* previously fed for 2 hrs radiolabelled phytoplankton (HET, EMI or ISO). A: mean remaining activity (%) ± SD.

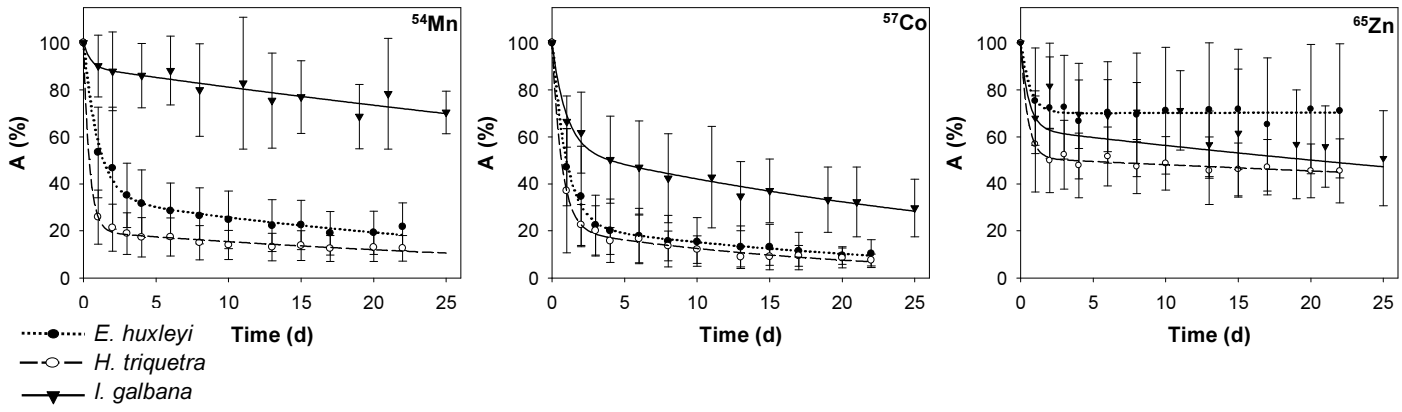
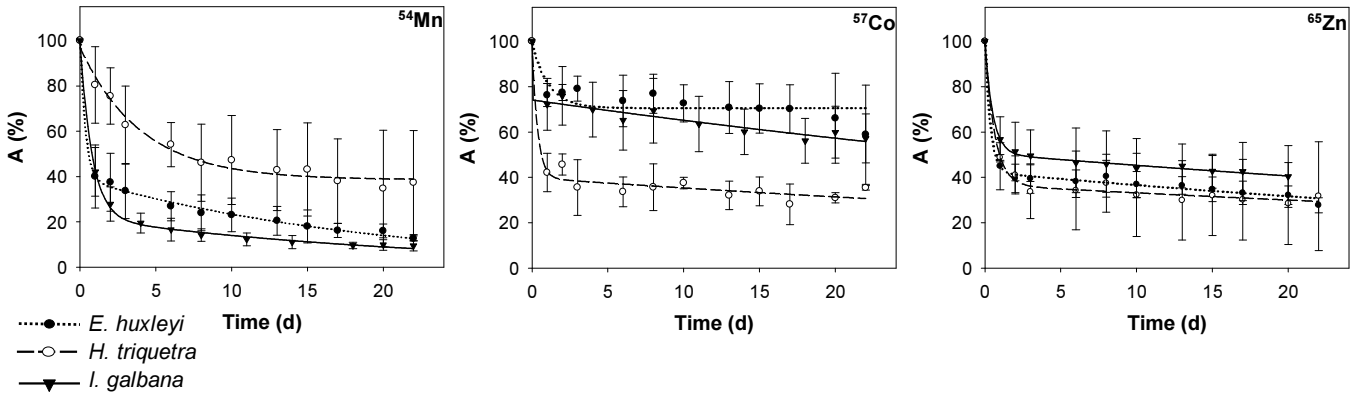


Figure 5. Influence of phytoplankton strains (*Heterocapsa triquetra*, HET, *Emiliania huxleyi*, EMI and *Isochrysis galbana*, ISO; 10^4 cell ml^{-1}) on whole-body loss kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn in the clam *Gafrarium tumidum* previously fed for 2 hrs radiolabelled phytoplankton (ISO, HET or EMI).

A: mean remaining activity (%) ± SD.



IV. DISCUSSION

During the last decades, dietary pathway has been increasingly recognized as a major source for contaminant uptake in marine organisms (e.g. Wang et al. 1996; Reinfelder et al. 1998; Wang & Fisher 1999b). The assimilation efficiency (AE) and retention time ($T_{b½}$) being the critical parameters in assessing and modeling the dietary uptake of contaminants, numerous studies have been devoted to assess the formers in different marine organisms (e.g. Warnau et al. 1996b; Wang & Fisher 1999a; Warnau et al. 1999). However, few data are available for tropical organisms (Phillips 1991) and, to the best of our knowledge, none are available for the species investigated in the present study.

Ideally, the concentrations of metals in the tissues of a bioindicator species should reflect those occurring in the ambient environment. This relevant criterion has been previously experimentally assessed for *I. isognomon* and *G. tumidum* exposed to the range of dissolved concentrations of As, Cr, Co, Cd, Mn, Ni and Zn that is encountered in the New Caledonia lagoon waters (Hédouin et al. submitted-b; Hédouin et al. submitted-d). These studies indicated that these elements are bioconcentrated in both bivalves in direct proportion to their

dissolved concentration in seawater and hence that body concentrations are directly reflecting concentrations in seawater. The experimental results presented here are complementary with these previous studies as they broaden the available knowledge to the dietary pathway of metal bioaccumulation. Presented data demonstrated that Co ingested with phytoplankton previously exposed to a range of Co concentration (up to 500 ng added Co l^{-1}) was assimilated in the same proportion and retained similarly whatever the food-associated Co concentration. The experimental conditions were designed to cover the whole range of Co concentrations that can be encountered in New Caledonian waters from pristine to extremely contaminated areas (Goro-Nickel 2004). Similar trends have been previously reported by Chong & Wang (2000) who observed that metal concentration in sediment had little effect on the assimilation efficiency of sediment-bound metals in the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. Wang & Fisher (1996) showed that the response of the mussel *Mytilus edulis* to metal concentration variation in ingested food was dependent on the element investigated. Indeed, AE for Se was not affected by the concentration in the ingested diatoms (*Thalassiosira pseudonana*) whereas AE for Zn and Cd respectively decreased and increased when the diatom food was more contaminated with Zn and Cd. The present results on Co AE in *G. tumidum* and *I. isognomon* are concordant with those obtained from exposure of the same species to Co dissolved in seawater (Hédouin et al. submitted-b). Bioconcentration and retention capacities for these elements were generally not affected by concentrations in seawater (over two to three orders of magnitude). Nevertheless care should be taken in generalising Co results to other metals, and further studies should specifically investigate the dietary behaviour of other major contaminants in the New Caledonian lagoon.

In this study, whereas food quality and quantity were shown to have limited influence on the retention time of metals in organism tissues, the assimilation efficiency of investigated metals generally differed according to the feeding condition. Metals were often better assimilated when bivalves were fed *I. galbana* than the two other phytoplankton strains (*H. triquetra* or *E. huxleyi*). *I. galbana* has comparable cell length (c.l.) and cell width (c.w.) (c.l.: 4-6 µm; c.w.: 2-4 µm) than *E. huxleyi* (c.l.: 3-4 µm; c.w.: 3-4 µm), but is much smaller than *H. triquetra* (c.l.: 20-28 µm; c.w.: 14-18 µm). However, the differences and similarities in AE observed among the strains indicate that phytoplankton size would not be the major factor responsible for the variation in metal AE in the two bivalves. Bivalves are able to feed selectively on particles of different size and of different nature (Newell et al. 1989) and species-related

selectivity and/or dietary preferences could more probably explain the specific differences observed in AE.

Food availability is another key factor that is well known to influence feeding behaviour of filter-feeding bivalves (e.g. Bayne et al. 1987; Bayne 1993). Generally, filter-feeders can adjust their ingestion rate to ambient phytoplankton density and thereby are able to maintain a stable ingestion rate even at high food concentrations (Jin et al. 1996; Dong et al. 2000; Zhuang & Wang 2004). Although no conclusion on the influence of this adaptative feeding behaviour could be directly drawn from our results, it is clear that the food availability notably influenced metal AE in the bivalves studied. Hence, taking into account the feeding behaviour of organisms according to feeding conditions is essential to better apprehend the prediction of dietary metal uptake using bioaccumulation models (Thomann et al. 1995) and to interpret bioaccumulation data obtained in the framework of biomonitoring programmes. For example, Bendell-Young & Arifin (2004) demonstrated the influence of mussel feeding behaviour on their predicted tissue concentrations in Cd, especially under conditions of highly variable quantity and quality of suspended particles.

Overall, our experimental results suggest that food quality and quantity may play a significant role in the assimilation of metals ingested with food in *I. isognomon* and *G. tumidum*. Due to the now recognized importance of dietary contribution to global metal bioaccumulation in marine organisms (Lee & Luoma 1998; Reinfelder et al. 1998), it is thus recommended to pay great attention to factors influencing AE. This would help refining both bioaccumulation model predictions and interpretation of field results from bioindicator based surveys and monitorings.

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DEUXIEME PARTIE
VALIDATION *IN SITU* DE LA VALEUR BIOINDICATIVE DES ESPECES

La caractérisation du potentiel bioaccumulateur des espèces en laboratoire constitue la première étape permettant de déterminer la valeur bioindicative des organismes sélectionnés, l'algue brune *L. variegata*, l'huître *I. isognomon* et *M. regula,* et le clam *G. tumidum.* Les expériences menées en laboratoire ont confirmées que les espèces sélectionnées étaient des espèces bioindicatrices intéressantes, et particulièrement dans le cas de l'algue brune *L. variegata* et des deux huîtres *I. isognomon* et *M. regula* de part leurs fortes capacités de bioaccumulation des contaminants. Cependant les paramètres cinétiques obtenus jusqu'à présent sont représentatifs de conditions contrôlées en laboratoire où l'influence de chaque paramètre est étudiée séparément. En milieu naturel, il existe diverses interactions entre un grand nombre de paramètres (température, salinité, turbidité…). Toutes ces variations environnementales peuvent influencer la bioaccumulation des contaminants dans les organismes. Des expériences *in situ* sont alors nécessaires pour étudier et caractériser le pouvoir bioaccumulateur des organismes sélectionnés en condition réelles ainsi que la dynamique *in situ* des processus d'accumulation et d'élimination des contaminants dans les organismes sélectionnés. Les deux huîtres *I. isognomon* et *M. regula* présentant un comportement bioaccumulateur relativement similaire vis-à-vis des différents contaminants étudiés (à l'exception de l'Ag), seule l'huître *I. isognomon* a été considérée dans la partie expérimentale *in situ*. Dans cette deuxième partie, les concentrations en 9 éléments (Ag, As, Cd, Co, Cr, Cu, Mn, Ni et Zn) ont été mesurées dans l'algue brune *L. variegata*, l'huître *I. isognomon* et le clam *G. tumidum*. L'approche expérimentale vise à déterminer si les espèces sélectionnées sont à même d'être utilisées comme des bioindicateurs de la contamination métallique dans des approches de biosurveillance active et/ou passive, et s'articule autour de deux thématiques :

- **l'étude de la variation géographique des concentrations en contaminants dans les trois espèces sélectionnées.**
- **l'étude des cinétiques d'accumulation et d'élimination des contaminants en milieu naturel (expériences de transplantation)**.

Les grands projets de biosurveillance existant à l'heure actuelle (e.g. Mussel Watch, Goldberg et al. 1983) collectent des organismes provenant de différentes stations et suivent l'évolution temporelle des concentrations en contaminants. Cependant, avant d'utiliser des organismes comme des bioindicateurs de la contamination dans des approches de biosurveillance passive, il est important de s'assurer que les concentrations en contaminants mesurées dans les organismes reflètent effectivement le degré de contamination des sites considérés. Ainsi, pour

cette étude, les trois espèces sélectionnées ont été collectées dans différents sites afin de vérifier leur aptitude à concentrer les contaminants de manière proportionnelle au degré de contamination du site dans lequel elles vivent.

Dans la deuxième thématique, des transplantations d'organismes de sites non contaminés vers des sites fortement contaminés, et *vice versa*, ont été réalisées afin de déterminer le potentiel bioaccumulateur et dépurateur des espèces sélectionnées en conditions contaminantes complexes. Dans la transplantation d'organismes de sites non contaminés vers des sites contaminés, deux aspects ont été considérés : la transplantation d'organismes dans des sites où les espèces résident naturellement, et dans des sites où les espèces n'existent pas naturellement. Ces expériences de transplantation apportent des informations très utiles concernant la future utilisation de ces espèces au titre de bioindicateurs dans une approche de biosurveillance passive/active. En effet, la biosurveillance active utilisant la transplantation d'organismes est une manière très efficace de suivre le degré de contamination des sites (de Kock & Kramer 1994). Le principal avantage de cette technique par rapport à la biosurveillance passive est la possibilité de sélectionner des sites indépendamment de la présence d'espèces résidentes.

Les résultats de cette deuxième partie sont rapportés sous formes de 3 articles scientifiques.

CHAPITRE 8

Concentrations de 9 éléments (Ag, As, Cd, Co, Cr, Cu, Mn, Ni et Zn) dans des algues et des bivalves tropicaux du lagon de Nouvelle-Calédonie : Étude des variations géographiques et des compartiments corporelles

Les concentrations en 9 éléments (Ag, As, Cd, Co, Cr, Cu, Mn, Ni et Zn) ont été mesurées dans l'algue brune *Lobophora variegata*, l'huître *Isognomon isognomon* et le clam *Gafrarium tumidum* provenant de différents sites le long de la côte Sud Ouest de la Nouvelle-Calédonie afin d'évaluer et valider leur utilité en tant qu'espèces bioindicatrices. Les résultats indiquent que les concentrations mesurées dans les trois organismes reflètent les différences géographiques des niveaux de contamination, préalablement établis à l'aide des analyses des éléments dans les sédiments. De plus, la station d'échantillonnage explique la majeure partie de la variabilité observée dans les concentrations mesurées. Par rapport aux mesures effectuées dans les organismes, deux des huit stations considérées peuvent être considérées comme des sites de références relatifs pour tous les éléments, à l'exception de l'As, pour lequel des niveaux très élevés ont été détectés dans les tissus des clams et des huîtres (jusqu'à 441 $\mu g\ g^{-1}$ poids sec pour les clams). En conclusion, les trois organismes tropicaux étudiés dans ce travail peuvent être utilisés comme des espèces bioindicatrices fiables pour surveiller le degré de contamination dans les eaux côtières de Nouvelle-Calédonie, avec des perspectives raisonnables d'application dans d'autres écosystèmes coralliens.

Concentrations of 9 selected elements (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn) in algae and bivalves from the New Caledonia lagoon: geographical and body compartment variations

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ABSTRACT. The concentrations of 9 elements (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn) were measured in the brown alga *Lobophora variegata*, the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* from different sites along the SW New Caledonian coast in order to assess their usefulness as bioindicator organisms. Results indicated that concentrations in the three organisms mirrored the geographical differences in contamination levels as established through element analyses in sediment. Moreover, sampling location explained the major part of the variability in element concentrations. On the basis of organism analyses, two out of the eight investigated stations can be considered as relative "reference" sites, except for As, for which very high levels were detected in clam and oyster tissues (up to 441 μg g^{-1} dry wt for clams). In conclusion, the three tropical organisms investigated could be used as valuable bioindicator species for surveying metal contamination in the coastal waters of New Caledonia with reasonable perspectives of larger application to other coral reef environments.

Keywords: Tropical Environment, Mining activities, Bioindicator, Contamination

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I. INTRODUCTION

Surrounded by a barrier reef of 1600 km, the New Caledonia lagoon is one of the largest in the world (Labrosse et al. 2000). However, the lagoon of New Caledonia is subject to important anthropogenic inputs of metals mainly due to intense land-based Ni mining activities but also to urban development and lack of efficient wastewater treatment. Open-cast mining exploitation presently constitutes the major economical resource of the Territory and results in important coastal discharges of metals, which constitute a threat to coral reef ecosystems (Labrosse et al. 2000). Recently, more efficient extraction processes based on acidic extraction (viz. lixiviation) have been developed (Mihaylov et al. 2000; Goro-Nickel 2001), making the extraction from low Ni grade ores (limonite) possible. The acidic extraction of metals is not Ni-selective and also solubilizes all other ore-contained by-product metals. Therefore, the lixiviation process will obviously lead to an increased multi-elemental contamination of the coastal marine environment.

Although mining activities are rising up in the island, studies reporting metal concentrations in tropical marine organisms from New Caledonia are scarce (Monniot et al. 1994; Bustamante et al. 2000; Breau 2003). In this context, development and implementation of metal monitoring programmes in the New Caledonia lagoon is a strong priority.

Among the approaches used to assess environmental contamination, the usefulness of bioindicator species is now well established. Marine organisms provide valuable information on the geographical and temporal variations of the bioavailable metal concentrations in their environment (Rainbow 1995; Warnau et al. 1998). Ideally, selected bioindicators should display a simple relationship between metals accumulated in their tissues and the ambient metal concentrations. This should be true regardless of location and environmental conditions (e.g. temperature, salinity) considered.

Algae and molluscs have been extensively used in temperate regions (e.g. Mussel Watch program; Goldberg et al. 1983; Rainbow 1995), whereas little attention has been paid to the identification of bioindicators specifically adapted to tropical and sub-tropical regions (Phillips 1991) despite the constant increase in industrial and human activities. Some efforts were devoted to the extension of the Mussel Watch to the Asia/Pacific region (UNU 1994), using bivalves such as *Saccostrea* spp., *Crassostrea* spp. and *Perna* spp. as bioindicators (Phillips 1985; Rainbow 1993a). However, none of the above-cited species is present in

sufficient abundance along the New Caledonia coasts to be considered as a useful candidate to monitor local contamination. Therefore, other tropical organisms have to be selected. In this context, a recent study screened metal concentrations in a variety of local marine organisms from different parts of the New Caledonian lagoon with supposed contrasting contamination status (Breau 2003). That first study showed that three species satisfied the basic requirements to be met by a bioindicator species (Moore 1966; Phillips 1990): the oyster *Isognomon isognomon*, the clam *Gafrarium tumidum* and the brown alga *Lobophora variegata*.

The aim of the present study was to assess the potential of these three species as sentinel organisms in tropical waters and to provide information on the degree of contamination of selected elements (Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Zn) in different locations along the SW coast of New Caledonia. Results presented in this paper also provide baseline data for future monitoring programs.

II. MATERIALS AND METHODS

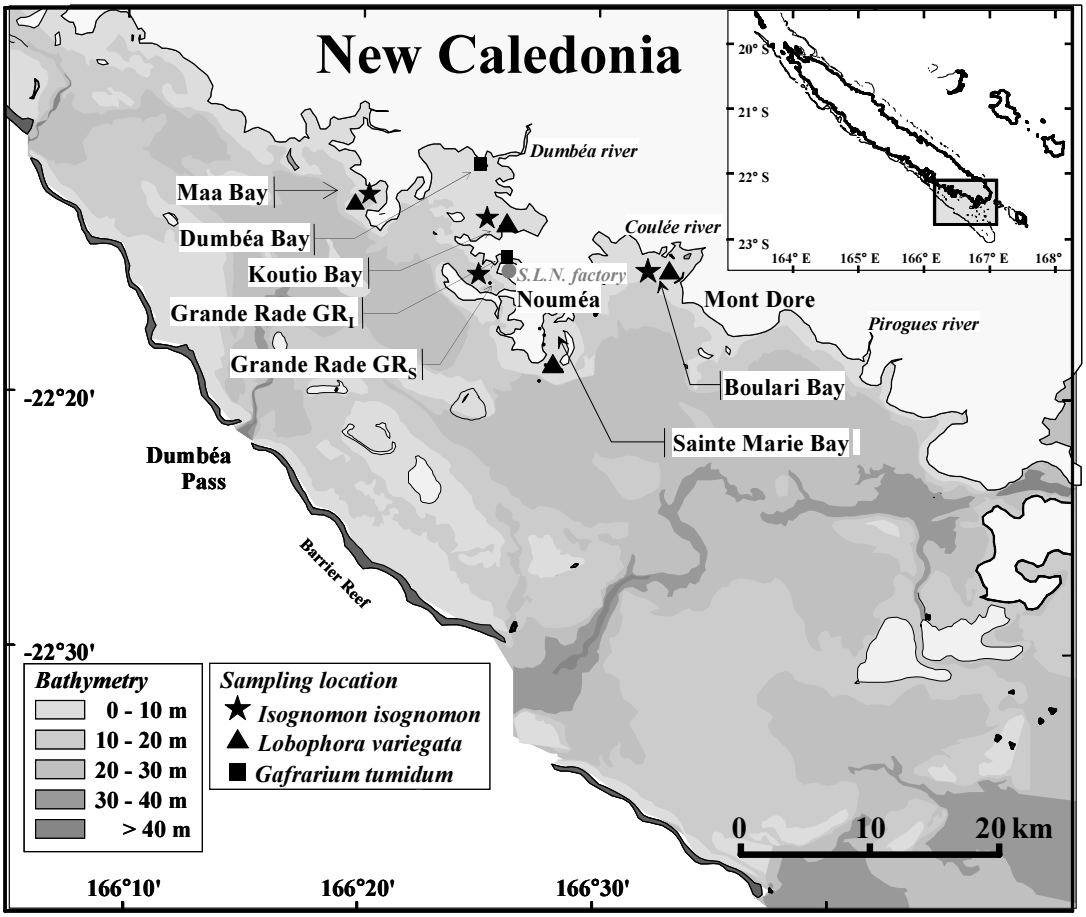
II.1. SAMPLING SITES

The sampling stations were selected according to supposedly contrasting contamination status (Fig. 1). Algae and oysters were collected in three common sampling stations (Maa Bay, Koutio Bay and Boulari Bay) and specifically in Grande Rade (GR_S) for the oysters and in Sainte-Marie Bay for the algae (Fig. 1). Maa Bay is subject to low anthropogenic and terrigenous inputs and was considered as the relative "reference" station for oysters and algae. Koutio Bay is influenced by inputs of domestic wastes of Nouméa city and by the presence of an important rubbish dump. Boulari Bay is under the influence of La Coulée River that delivers important inputs of lateritic materials to the lagoon due to soil erosion of closed mine sites. Sainte-Marie Bay is subject to high urban inputs (wastewater discharges and streaming waters) mostly in its Northern part. Grande Rade (GR_S) is subject to anthropogenic inputs from the Ducos industrial zone and the metallurgic factory "Société Le Nickel" (SLN).

For clams, three intertidal sampling stations were selected: Ouano Beach, Dumbéa Bay and Grande Rade (GR_I). Ouano Beach is situated 100 km northward from Nouméa, and is not influenced by industrial activities; it was considered as the "reference" station for clams. Grande Rade (GR_I) is subject to anthropogenic inputs from the SLN factory (scoria and waters), from the Shell Pacific factory (effluents) and from domestic discharges. Dumbéa Bay

is an estuarine bay, influenced by waters from La Dumbéa River and subject to terrigenous inputs.

Figure 1. Selected sampling locations along the SW coast of New Caledonia (Ouano Beach is not represented on the map).



II.2. ORGANISMS

The clam *Gafrarium tumidum* was collected by handpicking in the intertidal stations. The oyster *Isognomon isognomon* and the alga *Lobophora variegata* are associated with rocky substrata at depth ranging from 2 to 25 m and were collected by SCUBA diving.

All the organisms (n = 6 per species per station) were collected from October to November 2004 to reduce as much as possible the variability of element concentrations due to season or sexual cycle. Body size is well-known to affect metal concentrations in organisms (e.g.

Boyden 1977); therefore only samples with shell width longer than 35 mm for *G. tumidum* (Hédouin et al. in press) and shell length longer than 70 mm for *I. isognomon* (Metian 2003) were selected for analysis. Back to the laboratory, the bivalves were kept for 24 hrs in 30 l of seawater from the same sampling station to allow for depurating gut contents and particulate material present in the mantle cavity. Epiphytes and sediments were removed from algae by gentle scrubbing and thorough rinsing with clean seawater. Three body compartments for the clams (digestive gland, gills and remaining soft parts) and four body compartments for the oysters (visceral mass, gills, adductor muscle and remaining soft parts) were removed from the shells. The separated body compartments and alga samples were weighed (wet wt), dried at 60°C until constant weight, and weighed again (dry wt). They were then stored in acid-washed, hermetically sealed PET containers until analysis for their metal contents.

II.3. SEDIMENT SAMPLES

In parallel to organisms, superficial sediments (top 5-cm layer) were analysed in all the sampling stations (Fig. 1), except in Dumbéa Bay and in Grande Rade (GR_S) (samples lost during diving). Sediments were stored in acid-washed PET bags until return to the laboratory; they were then dried at 60°C for 5 d. In order to eliminate heterogeneous materials (e.g. stones, fragment of corals), sediments were sieved (1-mm mesh size) prior to analysis for their metal contents.

II.4. SAMPLE PREPARATION AND ANALYSES

Aliquots of the biological samples (0.1 to 0.5 g) and of the sediment samples (0.5 g) were digested using a 3: 1 (v: v) 65 % HNO_3 - 30 % HCl mixture (Merck, suprapur quality). Acidic digestion of the samples was carried out overnight at room temperature, then using a MARS V microwave (30 min with constantly increasing temperature up to 100°C for sediment and up to 115°C for biota, then 15 min at this maximal temperature). Each sample was eventually diluted to 30 to 50 ml with milli-Q water according to the amount of sample digested.

Elements were analysed using a Varian Vista-Pro ICP-OES (As, Cr, Cu, Mn, Ni, and Zn) or a Varian ICP-MS Ultra Mass 700 (Ag, Cd and Co). Three control samples (two Certified Reference Materials -CRM- and one blank) treated and analysed in the same way as the samples were included in each analytical batch. CRM were dogfish liver DOLT-3 (NRCC) and lobster hepatopancreas TORT-2 (NRCC). The results for CRM indicated recoveries ranging from 79 % (Cr) to 122 % (Ni) (Table 1). The detection limits were 10.1 (As), 0.8

(Cr), 0.5 (Cu), 0.04 (Mn), 1.1 (Ni) and 0.7 (Zn) µg g^{-1} dry wt for ICP-OES and 0.1 (Ag), 0.3 (Cd) and 0.03 (Co) µg g^{-1} dry wt for ICP-MS. Mean element concentrations are given on a dry weight basis (µg g^{-1} dry wt).

Table 1. ICP-OES and ICP-MS analysis of Certified Reference Materials (TORT-2 and DOLT-3): certified and measured values (mean ± SD, µg g^{-1} dry wt, n = 6) (n.c.: value not certified).

Elements	Method	TORT-2			DOLT-3		
		Measured	***Certified***	**%**	***Measured***	***Certified***	**%**
		Mean ± SD	*Mean ± SD*	**Recovery**	*Mean ± SD*	*Mean ± SD*	**Recovery**
Ag	**ICP-MS**	6.01 ± 1.78	n.c.		1.21 ± 0.09	1.20 ± 0.07	103
As	**ICP-OES**	21.2 ± 3.6	21.6 ± 1.8	98	9.9 ± 0.3	10.2 ± 0.5	97
Cd	**ICP-MS**	25.4 ± 3.0	26.70 ± 0.60	105	19.98 ± 1.12	19.40 ± 0.60	100
Co	**ICP-MS**	0.45 ± 0.09	0.51 ± 0.09	112	0.29 ± 0.05	n.c.	
Cr	**ICP-OES**	0.61 ± 0.16	0.77 ± 0.15	79	4.02 ± 0.93	n.c.	
Cu	**ICP-OES**	91.0 ± 16.5	106.0 ± 10.0	86	32.1 ± 0.6	31.2 ± 1.0	103
Mn	**ICP-OES**	11.4 ± 2.0	13.6 ± 1.2	84	9.69 ± 0.1	n.c.	
Ni	**ICP-OES**	2.57 ± 1.26	2.50 ± 0.19	103	3.31 ± 0.5	2.72 ± 0.35	122
Zn	**ICP-OES**	171.1 ± 29.8	180.0 ± 6.0	95	100.7 ± 1.4	86.6 ± 2.4	116

II.5. STATISTICAL ANALYSES

Comparisons of the data were performed using 1- or 2-way analysis of variance (ANOVA) followed by the multiple comparison test of Tukey (Zar 1996). Two-way ANOVA was used with sampling location and body compartment as fixed factors. The variability explained by each factor and their interaction was derived from the sum of squares (Zar 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

III.1. SEDIMENTS

Table 2 shows the element concentrations measured in the sediment collected in the different stations. Except for Ag, for which comparison among stations was not possible due to concentrations always under the detection limit (Table 2), statistical analyses indicated contrasting element concentrations among stations. Boulari Bay and Grande Rade (GR_I) displayed the highest concentrations for all elements (Table 2). Concentrations in all elements were always significantly higher in Grande Rade (GR_I) than in Ouano Beach ($p_{Tukey} < 0.0001$). Concentrations in sediment from Boulari Bay were significantly higher (up to 1 order of magnitude) than those measured in the other stations where algae and oysters were also sampled.

III.2. THE ALGA *LOBOPHORA VARIEGATA*

Cr, Mn, Ni and Zn concentrations measured in *L. variegata* varied significantly among stations ($p_{Tukey} \leq 0.001$; Table 2). The lowest concentrations were measured in algae from Maa Bay (from 8 to 33 $\mu g\ g^{-1}$ dry wt according to the element considered) and the highest ones in Boulari Bay (from 62 to 150 $\mu g\ g^{-1}$ dry wt). Differences among stations were also found for Cu ($p_{Tukey} \leq 0.001$) which showed the lowest concentrations in Sainte-Marie Bay (1.1 $\mu g\ g^{-1}$ dry wt) and the highest ones in Boulari Bay (5.9 $\mu g\ g^{-1}$ dry wt) (Table 2). In contrast, no significant difference was observed for Ag, As and Co in the four sites. Ag and Cd displayed concentrations always lower than 1 $\mu g\ g^{-1}$ dry wt.

III.3. THE OYSTER *ISOGNOMON ISOGNOMON*

Among the two factors considered (body compartment and sampling location) and their interaction, the sampling location explained the major part of the variability observed for As, Cd, Cr and Ni (accounting for 16 to 70 % of the global variance) (Tables 3 and 4, Fig. 2A) whereas the body compartment was the predominant factor explaining the variability observed for Cu (29 %), Mn (49 %) and Zn (36 %). Significant interaction between body compartment and sampling location factors was detected for all elements except Mn, and accounted for 14 to 44 % of the global variance, indicating that geographical variation of measured concentrations was dependent upon the body compartment considered. For all

elements, an important part of the variation was associated with the residual term, ranging from 12 to 58 %, indicating that other, non-investigated factors (biological and/or environmental factors) were also influencing metal concentrations in the oyster soft tissues.

Table 2. Element concentrations in sediment and in the alga *Lobophora variegata* (mean ± SD; µg g^{-1} dry wt, n = 3 for sediment and n = 6 for algae).

Sampling stations	Ag	As	Cd	Co	Cr	Cu	Mn	Ni	Zn
Sediment									
Ouano Beach [1]	< 0.1*	2.9 ± 1.2 [a]	< 0.3*	0.8 ± 0.4 [a]	7.2 ± 2.7 [a]	0.5 ± 1.0 [a]	41.7 ± 15.7 [a]	5.1 ± 3.2 [a]	3.3 ± 1.9 [a]
Grande Rade GR_I [1]	0.4 ± 0.1	8.5 ± 1.6 [b]	2.4 ± 0.3	46.2 ± 9.1 [b]	292 ± 56 [b]	26.9 ± 8.8 [b]	288 ± 40 [b]	797 ± 149 [b]	141 ± 18 [b]
Sainte-Marie Bay [2]	<0.1*	5.8 ± 0.2 [a]	< 0.3*	1.9 ± 0.1 [a]	15.0 ± 0.1 [a]	0.8 ± 0.1 [b]	32.9 ± 3.0 [a]	11.9 ± 0.1 [a]	3.9 ± 0.1 [a]
Maa Bay [2]	< 0.1*	6.4 ± 0.3 [a]	1.0 ± 0.2 [a]	4.6 ± 2.3 [ab]	44.1 ± 7.9 [a]	10.7 ± 3.5 [a]	132 ± 7.8 [b]	64.2 ± 13.5 [ab]	15.2 ± 3.1 [b]
Koutio Bay [2]	< 0.1*	9.9 ± 0.7 [b]	0.5 ± 0.01 [b]	6.2 ± 1.1 [b]	38.6 ± 2.7 [a]	1.1 ± 0.3 [b]	81.6 ± 3.5 [c]	82.0 ± 5.1 [b]	9.4 ± 0.6 [ab]
Boulari Bay [2]	< 0.1*	46.9 ± 1.5 [c]	5.1 ± 0.5 [c]	61.2 ± 16.2 [c]	662 ± 50 [b]	4.6 ± 1.6 [a]	565 ± 15 [d]	900 ± 78 [c]	33.1 ± 3.4 [c]
L. variegata									
Sainte-Marie Bay	0.23 ± 0.14 [a]	25.0 ± 7.9 [a]	0.14 ± 0.07 [a]	2.97 ± 1.51 [a]	12.4 ± 10.1 [a]	1.10 ± 0.41 [a]	45.7 ± 36.8 [a]	20.2 ± 18.4 [a]	15.7 ± 12.7 [a]
Maa Bay	0.36 ± 0.08 [a]	27.9 ± 1.8 [a]	0.24 ± 0.04 [b]	3.52 ± 1.00 [a]	7.8 ± 4.1 [a]	1.25 ± 0.34 [a]	33.4 ± 12.7 [a]	11.2 ± 6.8 [a]	14.4 ± 1.7 [a]
Koutio Bay	0.62 ± 0.84 [a]	26.6 ± 9.7 [a]	0.15 ± 0.05 [a]	3.30 ± 1.67 [a]	15.1 ± 7.9 [a]	1.37 ± 0.85 [a]	51.0 ± 24.4 [a]	33.0 ± 24.8 [a]	14.5 ± 7.9 [a]
Boulari Bay	1.06 ± 0.38 [a]	37.1 ± 5.6 [a]	0.30 ± 0.07 [b]	5.11 ± 1.33 [a]	62.3 ± 29.3 [b]	5.91 ± 1.32 [b]	136 ± 36 [b]	116 ± 52 [b]	150 ± 21 [b]

* Element concentrations < to ICP – MS detection limits

Significant differences among the mean concentrations measured in algae or sediments are indicated by superscripts; means sharing the same superscript are not significantly different from each other ($p_{Tukey} > 0.05$).

Comparisons among sediment concentrations were carried out separately among the stations where clams were collected ([1] superscript) and among those where oysters and algae were collected ([2] superscript).

Table 3. Element concentrations in the oyster *Isognomon isognomon* (mean ± SD; µg g^{-1} dry wt, n = 6).

Body compartments: G (gills), M (adductor muscle), VM (visceral mass), R (remaining soft parts), WSP (whole-soft parts; reconstructed values).

Compartments	% weight	Ag	As	Cd	Co	Cr	Cu	Mn	Ni	Zn
Koutio Bay										
VM	51 ± 12	21.7 ± 24.3	21.7 ± 5.2	1.13 ± 0.42	1.05 ± 0.65	13.6 ± 2.0	3.0 ± 2.2	26.3 ± 9.8	4.6 ± 3.4	3,983 ± 2,555
M	26 ± 6	2.4 ± 2.1	20.5 ± 2.9	1.23 ± 0.56	0.15 ± 0.04	2.2 ± 1.7	1.1 ± 0.3	4.4 ± 1.7	1.0*	1,356 ± 876
G	6 ± 2	52.7 ± 23.5	24.2 ± 6.6	1.47 ± 0.63	1.04 ± 0.35	7.1 ± 2.0	8.7 ± 2.7	8.8 ± 3.6	6.9 ± 2.4	11,357 ± 5,953
R	17 ± 5	9.6 ± 10.7	25.2 ± 5.8	1.59 ± 0.74	0.62 ± 0.34	5.9 ± 1.6	5.7 ± 3.2	30.3 ± 25.3	5.1 ± 1.5	6,346 ± 4,224
WSP		14.5 ± 7.1 [a]	21.6 ± 2.4 [a]	1.23 ± 0.40 [a]	0.69 ± 0.20 [a]	9.0 ± 1.6 [c]	3.1 ± 0.9 [a]	20.4 ± 8.3 [a]	3.6 ± 1.1 [a]	3,832 ± 1,529 [b]
Maa Bay										
VM	51 ± 4	2.04 ± 1.81	64.3 ± 9.9	1.97 ± 1.04	0.58 ± 0.26	3.1 ± 0.8	10.6 ± 1.6	17.1 ± 6.4	2.2 ± 1.1	11,333 ± 5,904
M	25 ± 1	0.12 ± 0.08	106 ± 13	0.81 ± 0.50	0.03 ± 0.03	2.7 ± 0.1	0.4 ± 0.3	4.7 ± 3.9	0.2*	5,781 ± 3,299
G	4 ± 2	0.58 ± 0.31	91.5 ± 20.6	3.66 ± 1.79	0.53 ± 0.14	4.8 ± 0.2	14.0 ± 5.0	13.0 ± 5.3	3.8 ± 0.8	41,790 ± 14,629
R	20 ± 7	0.12 ± 0.08	57.9 ± 9.5	3.01 ± 2.81	0.50 ± 0.001	4.5 ± 0.6	6.6 ± 0.7	55.3 ± 29.6	4.0 ± 0.3	17,694 ± 8,103
WSP		1.47 ± 1.09 [b]	76.6 ± 9.3 [b]	1.80 ± 1.4 [a]	0.45 ± 0.16 [a]	3.5 ± 0.5 [ab]	6.8 ± 0.5 [ab]	22.3 ± 14.6 [a]	2.2 ± 0.5 [a]	13,817 ± 6,621 [a]
Grande Rade (GR_S)										
VM	28 ± 2	37.1 ± 9.6	39.5 ± 4.5	1.21 ± 0.69	1.38 ± 0.25	3.8 ± 0.5	44.6 ± 17.3	42.8 ± 9.6	8.8 ± 2.0	8,188 ± 2,757
M	25 ± 3	3.7 ± 2.8	46.0 ± 4.9	0.81 ± 0.19	0.13 ± 0.04	0.6 ± 0.1	1.6 ± 0.3	5.0 ± 2.7	0.6*	1,958 ± 443
G	7 ± 2	217 ± 83	42.4 ± 8.8	0.93 ± 0.28	1.37 ± 0.44	6.3 ± 1.2	13.5 ± 3.5	8.7 ± 3.6	8.3 ± 2.7	14,360 ± 3,503
R	40 ± 3	18.3 ± 7.0	31.7 ± 4.1	1.46 ± 0.59	0.40 ± 0.14	2.5 ± 0.6	8.8 ± 2.7	52.0 ± 21.1	3.1 ± 0.5	10,233 ± 3,724
WSP		32.8 ± 6.5 [c]	38.2 ± 4.3 [c]	1.18 ± 0.44 [a]	0.67 ± 0.09 [b]	2.7 ± 0.3 [b]	17.3 ± 5.3 [c]	34.7 ± 11.5 [a]	4.4 ± 0.8 [a]	7,873 ± 2,087 [a]
Boulari Bay										
VM	28 ± 6	49.4 ± 8.4	59.2 ± 19.2	1.48 ± 0.88	3.29 ± 1.99	7.6 ± 2.9	24.8 ± 12.8	38.8 ± 21.8	26.0 ± 7.9	1,741 ± 2,175
M	21 ± 3	0.2 ± 0.2	56.5 ± 13.1	0.84 ± 0.37	0.24 ± 0.10	4.2 ± 0.5	1.0 ± 0.1	3.5 ± 1.4	1.7 ± 1.0	279 ± 94
G	8 ± 2	9.0 ± 5.9	51.2 ± 9.1	0.96 ± 0.50	2.56 ± 0.48	5.3 ± 1.4	7.0 ± 0.6	9.3 ± 3.7	29.8 ± 5.1	4,437 ± 1,880
R	43 ± 3	4.3 ± 3.1	45.9 ± 9.6	1.43 ± 0.78	1.18 ± 0.41	5.5 ± 5.1	6.3 ± 1.1	41.8 ± 21.9	14.5 ± 5.2	2,017 ± 1,427
WSP		16.5 ± 4.0 [a]	51.7 ± 10.8 [d]	1.28 ± 0.68 [a]	1.60 ± 0.49 [a]	5.7 ± 2.9 [a]	9.8 ± 2.1 [a]	30.8 ± 16.0 [a]	16.0 ± 3.7 [b]	1,718 ± 1,290 [b]

* Element concentration < to detection limits.

Differences among the mean concentrations measured in a given body compartment in the different locations are indicated by superscripts; means sharing the same superscript are not significantly different from each others ($p_{Tukey} > 0.05$)

III.3.1. Geographical variation

The sampling location significantly affected the concentrations of all studied elements in the body compartments of *I. isognomon* (2-way ANOVA, $p_{sampling\ locations} < 0.0001$), except for Mn ($p = 0.054$) (Table 4, Fig. 2A). Multiple comparison tests on the mean concentrations indicated that one sampling location displayed generally the highest concentrations for one or several elements, whereas the three other locations did not show significant difference in element concentrations, except for Mn (no difference among none of the sampling stations) and As (all stations significantly different from each others).

Table 4. Variability (%) in element concentrations measured in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* explained by the factors considered (body compartment and sampling location) and their interactions.

Factors	Explained Variability (%)								
	Ag	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Mn**	**Ni**	**Zn**
I. isognomon									
Compartment	20.1	6.6	12.6	27.1	19.6	28.8	49.2	19.6	35.5
Location	19.1	69.9	16.0	26.0	23.6	17.2	3.9	47.9	29.0
Compartment × location	43.6	8.9	14.0	14.3	28.2	34.6	7.2	20.8	17.2
Residual	17.1	14.7	57.5	32.6	28.6	19.3	39.7	11.7	18.4
G. tumidum									
Compartment	6.2	3.2	13.2	16.9	9.3	14.2	9.1	10.6	14.9
Location	39.1	84.1	30.1	23.1	38.6	61.6	22.5	39.4	13.3
Compartment × location	13.6	3.5	9.6	19.3	16.8	14.4	24.4	15.0	11.1
Residual	41.1	9.2	47.1	40.7	35.3	9.8	43.9	35.0	60.6

Concentrations of Co and Ni in the oysters were significantly higher in Boulari Bay than in Maa Bay ($p_{Tukey} \leq 0.001$; Table 3, Fig. 2A). Oysters from Grande Rade (GR_S) displayed significantly higher Ag and Cu concentrations than those measured in Maa Bay ($p_{Tukey} \leq 0.01$) whereas the highest Cr concentrations were measured in oysters from Koutio Bay ($p_{Tukey} \leq 0.05$). In contrast to all other elements, As and Zn concentrations were higher in oysters from Maa Bay.

Figure 2. Comparisons of element concentrations in bivalves, using multiple comparison tests of Tukey performed after 2-way ANOVA in (A) the oyster *Isognomon isognomon* and (B) the clam *Gafrarium tumidum*.

Mean concentrations are ranked from the left to the right by decreasing order. Concentrations in underlined body compartments or locations are not significantly different ($\alpha = 0.05$).

Body compartments: DG (digestive gland), G (gills), M (adductor muscle), VM (visceral mass) and R (remaining soft parts).

Sampling locations: OUA (Ouano beach), GR_S (Grande Rade, subtidal), GR_I (Grande Rade, intertidal) DUM (Dumbéa Bay), KOU (Koutio Bay) and SM (Sainte-Marie Bay).

A- *Isognomon isognomon*

A-1: Body compartment variation

Metal	Compartment ranking			
Ag	G	VM	R	M
As	M	G	VM	R
Cd	R	G	VM	M
Co	VM	G	R	M
Cr	VM	G	R	M
Cu	VM	G	R	M
Mn	R	VM	G	M
Ni	G	VM	R	M
Zn	G	R	VM	M

A-2: Geographical variation

Metal	Location ranking			
Ag	GR_S	KOU	BOU	MAA
As	MAA	BOU	GR_S	KOU
Cd	MAA	KOU	BOU	GR_S
Co	BOU	GR_S	KOU	MAA
Cr	KOU	BOU	MAA	GR_S
Cu	GR_S	BOU	MAA	KOU
Mn	GR_S	BOU	MAA	KOU
Ni	BOU	GR_S	KOU	MAA
Zn	MAA	GR_S	KOU	BOU

B- *Gafrarium tumidum*

B-1: Body compartment variation

Metal	Compartment ranking		
Ag	G	DG	R
As	DG	G	R
Cd	DG	R	G
Co	G	DG	R
Cr	DG	G	R
Cu	DG	G	R
Mn	DG	R	G
Ni	DG	G	R
Zn	DG	G	R

B-2: Geographical variation

Metal	Location ranking		
Ag	GR_I	DUM	OUA
As	OUA	GR_I	DUM
Cd	GR_I	OUA	DUM
Co	DUM	GR_I	OUA
Cr	GR_I	DUM	OUA
Cu	GR_I	DUM	OUA
Mn	GR_I	DUM	OUA
Ni	GR_I	DUM	OUA
Zn	GR_I	DUM	OUA

Geographical variation of the element concentrations in the whole-soft parts of the oysters (reconstructed data) were tested using 1-way ANOVA and Tukey test. Results were similar to those from the 2-way ANOVA performed on body-compartment specific concentrations, except for Cd and Zn. For these two latter elements, no significant difference was observed among whole soft parts in the four sites for Cd and among Maa Bay and Grande Rade (GR_S) for Zn (Table 3). The particular opposite pattern of As and Zn displaying highest concentrations in Maa Bay (up to 77 µg As g^{-1} dry wt and 13,817 µg Zn g^{-1} dry wt) was confirmed in whole soft parts data treatment.

III.3.2. Body distribution

Multiple comparison tests performed after 2-way ANOVA on the mean concentrations in each body compartment (all sampling locations together) indicated that the concentrations of all elements were lower in the adductor muscle than in the other body compartments (Fig. 2A). Generally, concentrations in gills and visceral mass were not significantly different, but significantly higher than in the other body compartments.

In terms of distribution of total element load among body compartments, visceral mass and remaining soft parts contained the highest proportion of the elements. Body distributions did not differ among sampling locations, except for Ag which occurred in higher proportion (43 %) in the gills of oysters from Grande Rade (GR_S) compared to those from the other stations (5 - 24 %).

III.4. THE CLAM *GAFRARIUM TUMIDUM*

Two-way ANOVA performed on the whole set of data indicated that, with the exception of Mn and Zn, the sampling location was the predominant factor affecting element concentrations, accounting for 23 to 84 % of the global variance (Tables 4 and 5, Fig. 2B). The ranking of sampling stations by order of decreasing concentration depended on the considered clam body compartment. In the case of Ag, Cd, Co, Cr, Mn, Ni and Zn, 35 to 61 % of the element concentration variability was due to undetermined factor(s) (residual term).

III.4.1. Geographical variation

The mean concentrations of all elements measured in clams varied significantly according to the sampling locations (2-way ANOVA, $p_{\text{sampling locations}}$ always ≤ 0.002) (Tables 4 and 5, Fig. 2B). Results showed significant differences between Ouano Beach and Grande Rade (GR_I) for Ag, Cd, Cr, Cu, Mn, Ni and Zn, with the highest concentrations always found in Grande

Table 5. Element concentrations in the clam *Gafrarium tumidum* (mean ± SD; µg g^{-1} dry wt, n = 6).

Body compartments: DG (digestive gland), G (gills), R (remaining soft parts), WSP (whole-soft parts; reconstructed values).

Compartments	% weight	Ag	As	Cd	Co	Cr	Cu	Mn	Ni	Zn
Dumbéa Bay										
DG	14 ± 4	4.6 ± 6.0	70.3 ± 34.3	0.21 ± 0.11	4.6 ± 1.8	8.4 ± 4.3	22.0 ± 9.0	14.5 ± 9.9	33.9 ± 14.2	105 ± 42
G	12 ± 3	0.49 ± 0.55	39.6 ± 13.1	0.20 ± 0.11	4.5 ± 2.0	2.5 ± 1.3	7.4 ± 3.0	25.2 ± 26.3	37.9 ± 13.5	76.7 ± 24.1
R	75 ± 3	1.1 ± 0.9	32.8 ± 8.1	0.16 ± 0.02	3.6 ± 0.7	4.6 ± 1.6	5.9 ± 1.1	34.0 ± 36.5	29.0 ± 6.8	55.2 ± 9.8
WSP		1.4 ± 1.1 [a]	37.4 ± 7.4 [a]	0.17 ± 0.03 [a]	3.8 ± 0.7 [a]	4.8 ± 1.3 [a]	7.9 ± 1.3 [a]	35.9 ± 43.5 [a]	30.2 ± 6.0 [a]	62.7 ± 10.2 [a]
Ouano Beach										
DG	14 ± 3	1.4*	606 ± 135	0.33 ± 0.04	1.8 ± 0.2	3.5 ± 1.1	14.6 ± 2.7	5.0 ± 2.5	9.2 ± 1.7	78.3 ± 10.4
G	11 ± 3	0.01*	516 ± 117	0.19 ± 0.09	1.8 ± 0.5	1.9 ± 1.5	6.4 ± 2.2	7.9 ± 2.9	14.1 ± 4.4	89.7 ± 27.9
R	76 ± 5	0.02*	360 ± 121	0.16 ± 0.06	1.0 ± 0.3	3.1 ± 2.8	4.4 ± 1.1	5.9 ± 1.6	7.1 ± 1.5	50.7 ± 8.2
WSP		0.02* [a]	441 ± 84 [b]	0.19 ± 0.04 [a]	1.1 ± 0.2 [b]	3.2 ± 2.2 [a]	5.6 ± 1.0 [a]	5.5 ± 1.5 [a]	8.1 ± 1.5 [b]	55.6 ± 7.8 [a]
Grande Rade (GR_I)										
DG	29 ± 6	51.5 ± 33.6	67.9 ± 14.6	1.30 ± 0.88	2.4 ± 1.4	10.7 ± .4	146 ± 37	324 ± 260	91.7 ± 45.8	282 ± 276
G	12 ± 2	89.4 ± 75.6	63.2 ± 18.5	0.21 ± 0.09	5.6 ± 3.6	12.1 ± 3.4	119 ± 40	27.9 ± 20.3	49.0 ± 32.0	123 ± 65
R	59 ± 7	16.3 ± 10.8	47.1 ± 16.2	0.52 ± 0.46	1.5 ± 1.0	5.8 ± 1.8	34.3 ± 17.5	93.4 ± 86.2	29.7 ± 9.6	74.5 ± 12.7
WSP		33.1 ± 13.4 [b]	55.0 ± 15.1 [a]	0.74 ± 0.25 [b]	2.2 ± 1.0 [c]	8.0 ± 1.7 [b]	77.3 ± 17.5 [b]	139 ± 104 [b]	52.3 ± 11.9 [c]	154 ± 102 [b]

* Element concentration < to detection limits.

Differences among the mean concentrations measured in a given body compartment in the different locations are indicated by superscripts; means sharing the same superscript are not significantly different from each others ($p_{Tukey} > 0.05$)

Rade (GR_I). In contrast the highest concentrations of As were measured in Ouano Beach and were significantly different from those observed in all other locations ($p_{Tukey} \leq 0.001$; Table 5 and Fig. 2B).

Geographical variations were tested using 1-way ANOVA and Tukey test for the reconstructed element concentrations in the whole-soft parts of the clams (Table 5). Results were similar to those previously obtained with 2-way ANOVA performed on body-compartment specific concentrations, except for Co which showed significant differences among whole soft parts in the three sampling locations ($p_{Tukey} < 0.05$). Similarly to oysters, As levels in clams were highest in the "reference station" Ouano Beach, reaching values up to 441 $\mu g\ g^{-1}$ dry wt.

III.4.2. Body distribution

The mean concentrations of all elements investigated differed according to the body compartments (2-way ANOVA, $p_{body\ compartment}$ always ≤ 0.003). Multiple comparison tests of Tukey indicated that the concentrations of Cd, Cu, Cr, Mn and Zn were significantly higher in the digestive gland than in the other tissues ($p < 0.05$; Fig. 2B). Ag, As, Co and Ni concentrations were similar in the digestive gland and the gills. No major difference was found when considering body distribution in clams collected from Ouano Beach and Dumbéa Bay. In these two stations, the remaining soft parts contained the main fraction (55 to 77 %) of the total body burden for all elements. In contrast, in Grande Rade (GR_I), the elements were similarly distributed between the remaining soft parts and the digestive gland.

III.5. DIFFERENCE IN BIOACCUMULATION CAPACITIES OF THE THREE SPECIES INVESTIGATED

Statistical comparisons were performed among the investigated species in the stations were at least two of them were present (Fig. 1). In Boulari Bay, Ag, As, Cd, Cu and Zn concentrations were significantly higher in oysters than in algae whereas the concentrations of Co, Cr, Mn and Ni were lower ($p_{Tukey} \leq 0.01$; Tables 2 and 3). Similar results were obtained for oysters and algae from Koutio Bay ($p_{Tukey} \leq 0.004$), except for As and Cr whose concentrations were not significantly different between the two species. In Maa Bay, oysters displayed higher concentrations than algae ($p_{Tukey} \leq 0.006$) for As, Cu, Zn, and lower concentrations for Co ($p_{Tukey} \leq 0.02$). For the other elements (Ag, Cd, Cr, Mn, Ni), no significant difference was found between the two species.

Clams and oysters have been collected in two stations in Grande Rade (GR_I and GR_S, respectively). Significant differences between the concentrations measured in clams and oysters were found for all elements ($p_{Tukey} \leq 0.04$), except Ag and Cd (Tables 3 and 5). When significant differences were observed, concentrations in clams were always higher than in oysters, except for Zn which displayed higher concentrations in oysters.

IV. DISCUSSION

As sediments are a sink for marine contaminants (e.g. Salomons et al. 1987), measurement of their element concentrations is often carried out to assess the contamination status of the marine environment. Accordingly, Boulari Bay and Grande Rade (GR_I) may be considered as highly contaminated stations compared to Ouano Beach and Maa Bay. In turn, the two latter ones may be defined as relatively non-contaminated stations (see Table 2). However, it is now well known that sediment-associated concentrations are not necessarily representative of the contaminant fraction that is bioavailable for marine organisms, viz. the fraction of "direct ecotoxicological relevance" for these organisms (Phillips & Rainbow 1993). Therefore, the present study was carried out to assess the usefulness of the brown alga *Lobophora variegata,* the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* as sentinel species for Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn contamination in the SW lagoon of New Caledonia.

In agreement with sediment analyses, Maa Bay can also be considered as a relative reference site when considering element measurements in the alga *L. variegata* and the oyster *I. isognomon* for all elements, except As and Zn in oysters. The low element concentrations reported in this bay for algae and oysters are in the same range than those reported in the literature for *Isognomon* spp. and *Lobophora* spp. as well as in other oyster and alga genera from clean areas (see Tables 6 and 7). According to the element concentrations measured in algae, Boulari Bay may be considered as a highly contaminated station for all elements. Indeed, levels in algae were significantly higher (from several times to one order of magnitude) in Boulari Bay than in Maa Bay. In addition, the elevated concentrations of Co and Ni reported in oysters from Boulari Bay strongly suggest that a high degree of mining-related contamination occurs in Boulari Bay, most probably due to releases from surrounding closed mines and mining-enhanced erosion of the soils. This was further confirmed by the high concentrations of Co, Cr, Mn and Ni measured in the sediment from Boulari Bay. However, element analyses in oyster tissues indicated that other stations, not indicated

through sediment analysis, are also highly contaminated for some elements, especially Maa Bay for As and Zn and Grande Rade (GR_S) for Ag. The elevated concentrations recorded suggest that Maa Bay would be subject to agricultural activities and Grande Rade (GR_S) to important domestic wastewater discharges (Martin et al. 1988).

With the exception of As, element concentrations in the clam *G. tumidum* collected from Ouano Beach were lower than those found from Grande Rade (GR_I), which is in agreement with the results obtained from sediment analysis (see Tables 5 and 2, respectively). Concentrations measured in clams from Ouano Beach were in the same range as those reported for clean areas from other tropical zones (see Table 7). Ouano Beach may thus be considered as a relatively clean station for all elements considered, except for As. In contrast, Grande Rade (GR_I) can be defined as a highly contaminated station for Ag, Cr, Cu, Mn, Ni and Zn.

In this work, the distribution of the considered elements in bivalve tissues was also investigated in order to possibly identify some organs that could be more sensitive than others and able to respond more rapidly to changes of element contamination in the environment (Warnau et al. 1996b). Among the body compartments of clams, the digestive gland displayed the highest concentrating abilities. In addition, the concentrations measured in this organ easily allowed discriminating the stations according to their contamination levels. Hence, this organ could be proposed as a target for future biomonitoring programmes. In the oyster, no clear trends could be observed in bioaccumulation and geographical discrimination capacities among the different body compartments. Consequently, in future biomonitoring programmes, consideration of the whole soft parts of oysters could be recommended.

Table 6. Element concentrations (mean ± SD or range; µg g^{-1} dry wt) in brown algae from tropical and sub-tropical areas.

Species	Location	As	Cd	Co	Cr	Cu	Mn	Ni	Zn
Lobophora sp. [1]	Townsville Harbour, Australia		< 0.4			24.1		6.3	213
L. variegata [2]	Great Barrier Reef, Australia		0.28 - 0.4			0.74 - 1.6		0.85 - 1.5	2.6 - 5.5
Colpomenia sinuosa [3]	Aqaba, Jordan		7.8 ± 1.2			31.6 ± 3	21.4 ± 4.4		49.7 ± 8.7
C sinuosa [4]	Goa, India			10.56		21.06	258.15	23.03	221.5
Dictyopteris australis [4]	Goa, India			1.32 - 3.03		8.36 - 9.72	32.98 - 131.52	5.3 - 11.31	21.26 - 57.44
Dictyota bartayresii [5]	Penang Island, Malaysia		13.82			49.74	248.69		210
Ecklonia radiate [6]	South Australia, St Vincent gulf	50 - 145							
Enteromorpha intestinalis [7]	Tropical mangrove lagoon		0.26 ± 0.01	1.08 ± 0.12	3.8 ± 0.15	8.86 ± 3.25	114 ± 5		100 ± 3
Padina australis [2]	Great Barrier Reef, Australia					2 - 3		1 - 1.4	3.8 - 9.5
P. boergesenii [8]	Zanzibar channel, Tanzania	14.3 ± 0.84 - 34.1 ± 1.17	0.21 ± 0.01 - 2.66 ± 0.28		0.67 ± 0.04 - 8.81 ± 0.53	2.02 ± 0.1 - 16.2 ± 2.13	22.6 ± 0.67 - 461 ± 46.7	3.24 ± 0.17 - 9.37 ± 0.96	2.91 ± 0.4 - 47.1 ± 1.46
P. gymnosphora [9]	Porto Rico							23 - 32	
P. gymnosphora [10]	Tuticorin, India						20 - 1077		
P. pavonica [3]	Aqaba, Jordan, Red Sea		5.5 ± 1.1			41.6 ± 3.8	15.5 ± 0.9		92.7 ± 19.1
P. sanctae-crucis [11]	Abrolhos, Brazil				16.5 - 37.2	1.25 - 1.92	68 - 83	11.1 - 23.1	3.24 - 7.26
P. tenuis [5]	Penang Island, Malaysia		7.11		25.6	5.69	284		45.5
P. tetrastromatica [4]	Goa, India			4.35 - 8.58		3.17 - 7.86	206 - 531	8 - 18.3	4.5 - 11.7
P. tetrastromatica [12]	Goa, India	4.8 - 12.6				8.7 - 20.1	233 - 456		20.2 - 31.5
Sargassum bracteolosum [6]	South Australia, St Vincent gulf	64 - 123							
S. dentifolium [3]	Aqaba, Jordan		8.2 ± 1.2			42.1 ± 1.8	11.3 ± 2.1		41.8 ± 7.9
S. grevillei [5]	Penang Island, Malaysia		6.44		20.61	5.15	90.18		15.5
S. tenerrimum [12]	Goa, India	8.7 - 13.6				10.3 - 27.5	270 - 586		35.7 - 60.2
S; tenerrimum [3]	Goa, India			1.32 - 7.92		4.53 - 15.46	42.7 - 140.6	3.5 - 8.7	5.4 - 38.5

[1] Burdon-Jones et al. 1975, [2] Denton & Burdon-Jones 1986a, [3] Wahbeh et al. 1985, [4] Agadi et al. 1978, [5] Sivalingam 1978, [6] Maher 1983, [7] Szefer et al. 1998, [8] Engdahl et al. 1998, [9] Stevenson & Ufret 1966, [10] Ganesan & Kannan 1995, [11] Amado Filho et al. 1997, [12] Zingde et al. 1976

Table 7. Element concentrations (mean ± SD or range; µg g^{-1} dry wt) in clams and oysters from tropical and subtropical areas.

Species	Location	Ag	As	Cd	Co	Cr	Cu	Mn	Ni	Zn
Gafrarium tumidum [1]	Hong-Kong					0.67	5.77		5.59	57.5
G. tumidum [2]	Fidgi				1.0 - 2.8	1.0 - 1.6	4.2 - 11.0	28 - 45	1.7 - 4.5	
Anadara antiquate [2]	Fidgi				0.9 - 2.5	0.8 - 1.8	4 - 13	32 - 50	2 - 4	
Chione subrugosa [3]	Tropical mangrove lagoon			0.72 ± 0.09 - 2.25 ± 0.5	0.13 ± 0.14 - 1.1 ± 0.17	1.48 ± 0.28 - 1.93 ± 0.53	20.8 ± 1.49 - 23.4 ± 1.43	4.08 ± 0.21 - 4.55 ± 0.08	2.32 ± 0.35 - 2.65 ± 0.46	51 ± 4 - 73 ± 11
Circe sinensis [1]	Hong-Kong					2.26	3.13		2.8	43.7
Codakia orbicularis [4]	Dominican Republic			3.8		1.66	3.08		1.57	22.9
Ruditapes philippinarum [1]	Hong-Kong					0.9	3.99		4.66	98
Tellina fausta [4]	Dominican Republic			0.04		4.15	14.1		4.91	51.4
Circentia callipyga [5]	Gulf of Oman	3.03	156	1.17	4.45	0.97	8.35	17.7	23.9	69.1
Isognomon isognomon [6]	Phuket, Thailande						< 150			900 – 2,000
I. alatus [7]	Malaisia			0.47 ± 0.23 - 3.71 ± 0.12			11 ± 0.51 - 30.7 ± 0.8			23.8 ± 0.75 - 334.5 ± 12.5
I. alatus [8]	Venezuela			0.33 - 0.91		0.46 - 1.2	14 - 49.1		11 - 18	0.25 - 2.1
I. alatus [9]	Colombian Carribean			0.8 - 15.6			0.42 - 52.3			
I. alatus [4]	Dominican Republic			0.24 - 0.26		2.38 - 4.96	7.58 - 19.7		1.25 - 2.90	4,000 – 4,010
I. alatus [10]	Guadeloupe					0.23 - 7.2	6.8 - 127			1,060 – 12,163
I. alatus [10]	Martinique					0.32 - 1.75	5.4 - 248			2,459 – 11,527
I. bicolor [9]	Colombian Carribean			0.98 - 6.99			0.8 - 3.94			
I. legumen [11]	Taiwan						491 ± 29			
Isognomon sp. [12]	Biscayne Bay, Florida		37.3 ± 6.9							
Ostrea sandvicensis [13]	Hawaii						1,400		20	
Saccostrea amasa [14]	North Queensland, Australia			1 - 12						673 – 20,906
S. echinata [15]	North Queensland, Australia			0.69 - 2.34						2,080 ± 453
S. echinata [16]	North Queensland, Australia			0.198 - 4.63						325 – 4,680
Crassostrea belcheri [17]	Merbok estuary, Malaysia						1 - 8.5			30 - 550
C. cucullata [18]	Goa, India		2.3 - 6.3				251 - 728	33.2 - 17.5		446 – 2,800
C. echinata [14]	Cleveland bay, Australia									673 – 20,906
C. gryphoides [18]	Goa, India		3.2 - 5.8				175 - 210			325 - 550
C. iredalei [17]	Merbok estuary, Malaysia						4 - 8			80 - 550
C. gigas [19]	Derwent Estuary, Tasmania,									38,700
C. virginica [11]	Taiwan						257 ± 196			1,037 ± 778

[1] Cheung & Wong 1997, [2] Dougherty 1988, [3] Szefer et al. 1998, [4] Sbriz et al. 1998, [5] de Mora et al. 2004, [6] Brown & Holley 1982, [7] Saed et al. 2001, [8] Jaffe et al. 1998, [9] Campos 1988, [10] RNO-Antilles unpublished work, [11] Hung et al. 2001, [12] Valette-Silver et al. 1999, [13] O'Connor 1989, [14] Jones 1992, [15] Jones et al. 2000, [16] Olivier et al. 2002, [17] Lim et al. 1995, [18] Zingde et al. 1976, [19] Ayling 1974

The three investigated species accumulated some elements up to very high concentrations compared to the concentrations generally reported in the literature. The most interesting elements are discussed below.

Concentrations measured in algae bear out the high capacities of macroalgae to accumulate metals. Indeed, algae collected from Boulari Bay reached very high levels of Cr and Ni (62 ± 29 and 116 ± 52 μg g^{-1} dry wt, respectively) compared to the concentrations reported in the literature in other *Lobophora* species and in other brown algae (see Table 6). Likewise, Ni concentrations in clams from Grande Rade (GR_I) were higher (52 ± 12 μg g^{-1} dry wt) than those reported in the literature for other tropical clams (always lower by one order of magnitude, see Table 7). The high levels of Cr and Ni reported in this study in sediment and algae from Boulari Bay and in clams from Grande Rade (GR_I) are probably due to mining activities (presence of SLN industry in Grande Rade, which discharged its wastes into the Rade) associated with mining-enhanced erosion of lateritic soils, which are enriched in Cr and Ni (Labrosse et al. 2000).

Arsenic is one of the most toxic elements (Nriagu 1994) and was found to reach extremely high concentrations in the clams from Ouano Beach (441 ± 84 μg g^{-1} dry wt) compared to those observed in Grande Rade (GR_I) (55 ± 15 μg g^{-1} dry wt) and in the oysters from Maa Bay (77 ± 9 μg g^{-1} dry wt). Generally, As concentrations reported in the literature are lower than 30 μg g^{-1} dry wt for tropical and sub-tropical bivalves (e.g. Man & He 2000; Hung et al. 2001). Nevertheless, two studies on sub-tropical areas indicated elevated As concentrations in *Isognomon* species (37.3 ± 6.9 μg g^{-1} dry wt) (Valette-Silver et al. 1999) and in the clam *Circentia callipyga* from the Gulf of Oman (156 μg g^{-1} dry wt) (de Mora et al. 2004). However, to the best of our knowledge, such high concentrations of As have never been reported in other clam tissues. The reason for such high As concentrations is not clear. Sometimes, As concentrations in organisms were found to be related to the sediment concentrations like it has been reported for *Scrobicularia plana* (Langston 1980); however no similar correlation was observed here (Tables 2, 3 and 5). In addition, laboratory experiments have shown that bivalves generally displayed a limited capacity in accumulating As from seawater (e.g. mussel *Mytilus galloprovincialis*, Ünlü & Fowler 1979; clam *G. tumidum* and oyster *I. isognomon*, Hédouin et al. submitted-b). Thus, the elevated As concentrations reported in this study would be accumulated most probably from the diet of the organisms (Sanders et al. 1989). Accordingly, transfer along the food chain could be proposed as the main route of uptake for As, meaning that food of both oysters and clams are enriched in As

in Maa Bay and Ouano Beach, compared to the other sampling locations. Whereas some agricultural activities are conducted in Maa Bay, Ouano Beach is rather subject to waste discharges from shrimp aquaculture. Hence the latter important discharges of N-enriched products could locally modify the N:P ratio. In environments with phosphate deficit, it has been shown that phytoplankton metabolises As more easily. If this was to be the case here, this could result in an enhanced accumulation in bivalve tissues (Benson & Summons 1981). However, further investigations are needed to validate such a hypothesis in Maa Bay and Ouano Beach. Nevertheless, the extremely high As levels measured in clam tissues (441 ± 84 μg g^{-1} dry wt) are of considerable interest because (1) *G. tumidum* is a seafood product in New Caledonia and (2) little is known about the speciation of As within the tissues of *G. tumidum* (Francesconi et al. 1999) which determines its potential toxicity to consumers (Kaise & Fukui 1992).

I. isognomon displayed also very high Zn concentrations in Maa Bay and in Grande Rade (GR_S), viz. 13,817 ± 6,621 and 7,873 ± 2,087 μg g^{-1} dry wt, respectively. Elevated concentrations of Zn have been reported for *I. alatus*, reaching 4,010 μg Zn g^{-1} dry wt in individuals collected in the Dominican Republic (Sbriz et al. 1998) and 12,163 μg g^{-1} dry wt in the Guadeloupe (RNO-Antilles unpublished work). Although, this metal is well known to be essential to organisms, acting e.g. as a co-factor in numerous metalloenzymes (e.g., Vallee & Falchuk 1993), the amounts accumulated are clearly far above the physiological needs of the bivalve. *I. isognomon* must therefore possess a natural capacity to accumulate Zn up to very high levels while avoiding subsequent toxicity, such as the immobilization of Zn under non-toxic forms in granules which are very slowly excreted (e.g., Corrêa Junior et al. 2000). Indeed, in various bivalves and especially in oysters, granules may contain up to 60 % of the total body load of Zn (Eisler 1981). Even though Boulari Bay is proposed as the most contaminated location regarding Zn (algae and sediment displayed Zn levels up to 150 ± 21 and 33 ± 3 μg g^{-1} dry wt, respectively), the oysters from Maa Bay showed the highest Zn concentrations. Thus, the levels of Zn in the environment appear to be differently reflected by Zn concentrations in oysters and algae, suggesting that the simultaneous use of both organisms should be recommended to allow for gathering complementary information.

Ag is one of the most toxic metal (Ratte 1999) and the scarcity of data concerning Ag in tropical organisms is quite surprising. In this way, the concentrations measured in the three investigated species (Tables 2, 3 and 5) can be considered as baseline data for the New Caledonia lagoon as well as for other tropical environments. Clams and oysters collected from

Grande Rade displayed quite elevated Ag concentrations (33 ± 13 and 33 ± 7 μg g^{-1} dry wt, respectively), which are one to two orders of magnitude higher than those measured in bivalves from the clean stations (Ouano Beach or Maa Bay) and to the background concentrations generally considered for tropical areas (e.g. < 1 μg g^{-1} dry wt in mussels; Klumpp & Burdon-Jones 1982) and temperate areas (< 6 μg g^{-1} dry wt in clams and oysters; Cohen et al. 2001). Various bivalves are able to accumulate Ag up to very high concentrations by trapping it as Ag_2S, a stable and non-toxic compound (e.g., Berthet et al. 1992). The occurrence of a similar detoxification system in *G. tumidum* and *I. isognomon* could explain the high Ag concentrations observed in their soft tissues. Natural sources of Ag are quite rare in the environment (Luoma et al. 1995) and Ag is considered as a valuable proxy of anthropogenic inputs in coastal waters (Sanudo-Willhelmy & Flegal 1992). Therefore the enrichment of Ag in bivalves from Grande Rade would be most probably related to sewage sludge and industrial and boating activities.

V. CONCLUSIONS

In New Caledonia, contaminants released in the lagoon are clearly a matter of concern, as reflected by the high element concentrations found in the marine organisms investigated in the present work. The three species considered in this study merit consideration as bioindicator species in the New Caledonian tropical waters because of (1) their abundance and wide distribution in New Caledonia (as well as in other tropical areas), (2) their interesting bioaccumulation potential, and (3) their ability to reveal the differences in element concentrations among differently contaminated geographical areas.

The three tropical organisms showed a high degree of variability in their bioaccumulation potential for the different elements. These observations indicate that considering simultaneously several organisms to better describe the contamination of the marine environment may lead to difference in the assessment of bioavailable fractions (Rainbow & Phillips 1993). Whereas *L. variegata* would be proposed as an indicator of the contamination in the water column in a similar way as the alga *Ulva* spp. which is widely used in biomonitoring surveys in the Mediterranean Sea (e.g. Malea & Haritonidis 2000), the two bivalves would be proposed as indicators of the contamination characterizing also other compartments of the ambient environment (seawater, phytoplankton community and sediment-seawater interface). In future biomonitoring programmes in the SW lagoon of New

Caledonia, element concentrations in organisms from Ouano Beach and Maa Bay could be considered as background concentrations for all elements, except for As and Zn. Furthermore, due to the very high levels of As measured in clams from Ouano Beach, the speciation of As in clam tissues should be investigated in detail to determine whether they represent a potential hazard for consumers.

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CHAPITRE 9

L'algue brune tropicale *Lobophora variegata* en tant que bioindicateur de la contamination minière dans le lagon de Nouvelle-Calédonie : Transplantations *in situ*

De précédentes études de laboratoire et de terrain avaient identifié l'algue *Lobophora variegata* comme une espèce bioindicatrice potentiellement intéressante dans le lagon de Nouvelle-Calédonie, qui est sujet à une importante contamination métallique d'origine minière. Le but de ce travail était d'évaluer la valeur bioindicative de cette espèce sur le terrain en étudiant ses capacités bioaccumulatrices pour des contaminants-clés du lagon (Ag, As, Cd, Co, Cr, Cu, Mn, Ni et Zn). Des algues provenant d'un site propre ont été transplantées pour une période de 3 mois dans un site contaminé, et *vice versa*, afin de déterminer les cinétiques d'accumulation et de dépuration des 9 éléments sélectionnés. Les résultats ont montré que les concentrations en As, Cd, Co, Cr, Cu, Mn et Ni dans les algues transplantées dans un site contaminé augmentaient de manière linéaire avec le temps. En revanche, une élimination significative des contaminants n'a pas été observée pour les algues transplantées dans un site propre, à l'exception du Co.

L. variegata étant capable d'accumuler rapidement les contaminants avec le temps, et particulièrement les éléments d'importance majeure en Nouvelle Calédonie (viz. Co, Cr, Mn et Ni), elle apparaît par conséquent comme une excellente espèce bioindicatrice. Les résultats ont également fourni des informations de base nécessaires à l'utilisation de *L. variegata* dans des conditions expérimentales *in situ* ainsi que des informations quantitatives sur les niveaux de contamination métallique ambiant. La large distribution de *L. variegata* dans les régions tropicales permettra d'élargir son utilisation à d'autres écosystèmes côtiers tropicaux.

The tropical brown alga *Lobophora variegata* as a bioindicator of mining contamination in the New Caledonian lagoon: a field transplantation study

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To be submitted

ABSTRACT. Previous field and laboratory studies have identified the alga *Lobophora variegata* as a potentially valuable bioindicator species in the New Caledonia lagoon which is subject to important metal contamination due to intense land-based mining activities. The aim of this work was to further assess the bioindicative value of this species in the field by investigating its bioaccumulation capacity for local key contaminants (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn). Algae from a clean and a contaminated sites were cross transplanted for a period of 3 months in order to determine the *in situ* uptake and depuration kinetics of the 9 elements selected. Results indicated that algae transplanted to the contaminated site displayed a significant linear increase in concentrations with time for As, Cd, Co, Cr, Cu, Mn and Ni. In contrast, algae transplanted to the clean site did not show major depuration, except for Co.

L. variegata showed a rapid response time in metal uptake, especially for elements of major concern in New Caledonia (viz., Co, Cr, Mn and Ni) and would therefore be an excellent bioindicator species of metal contamination. The results also provide background scientific information necessary for using *L. variegata* under *in situ* experimental conditions so as to provide quantitative information on ambient metal contamination levels. The wide distribution of *L. variegata* in tropical areas further enhances its potential as a bioindicator species of metal contamination in various tropical coastal ecosystems.

Keywords: Metals, Tropical, Transplantation, Bioindicator, Alga

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I. INTRODUCTION

Most of the numerous studies dealing with bioaccumulation in aquatic systems focus on concentration levels in various species whereas very few have been conducted to test experimentally the validity of alleged bioindicator species (Rainbow 2002). When compared to temperate ecosystems and despite their increasingly acknowledged sensitivity to environmental stresses, tropical coral reef ecosystems are even more deprived of the essential scientific background information necessary to derive proper biological tools to be used in environmental monitoring of metal contamination. This is especially true in New Caledonia where economic development during the past century has been essentially based on Ni mining activities (New Caledonia is one of the major Ni ore deposit worldwide). Its huge coral reef lagoon is naturally influenced by natural soil erosion and associated metal inputs (Labrosse et al. 2000). Local mining activities result in important additional anthropogenic inputs of metals into the lagoon and thereby constitute a threat to the local coastal ecosystems (e.g., Bird et al. 1984; Laganier 1991; Ambatsian et al. 1997).

The usefulness of bioindicator species to monitor the degree of contamination in the marine environment is now well established (e.g., O'Connor 1998). Among marine organisms, brown macroalgae are known to efficiently bioaccumulate metals from their surrounding environment (e.g. Försberg et al. 1988; Phillips 1990). Therefore, macroalgae, and more particularly the Phaeophyceae family, have been used as indicators of metal contamination since the early seventies (Bryan 1983; Söderlund et al. 1988). However, quite less attention has been paid to the tropical and sub-tropical areas compared to temperate areas (e.g. Karez et al. 1994; Amado Filho et al. 1999).

In New Caledonia, a recent field study was conducted to screen metal concentrations and their geographical variations in a series of local species (Breau 2003). Among these species, the brown alga *Lobophora variegata* appeared as a very promising bioindicator candidate. Indeed, it is of reasonable size, sedentary, easily collectable and displays relatively high metal levels (Breau 2003; Hédouin et al. submitted-a). In addition, a recent experimental study using radiotracer techniques (Metian et al. submitted) demonstrated the bioconcentration and retention efficiency of the alga when exposed to a range of metal concentrations corresponding to those actually encountered in the New Caledonia lagoon. This study also indicated that the alga satisfied one of the most important pre-requisite for selecting a

bioindicator species: *L. variegata* concentrates Cd, Co, Cr, Mn, Ni and Zn in direct proportion to their dissolved concentrations in ambient seawater, with tissular concentrations thousands of times higher than those in seawater. Similarly, the retention efficiency of these metals was independent of the exposure concentration (Metian et al. submitted). Although this laboratory study carried out under controlled conditions undoubtedly provided essential information regarding the excellent potential of *L. variegata*, no information is available on metal bioaccumulation behaviour of the alga in ecological relevant conditions, viz. in the field.

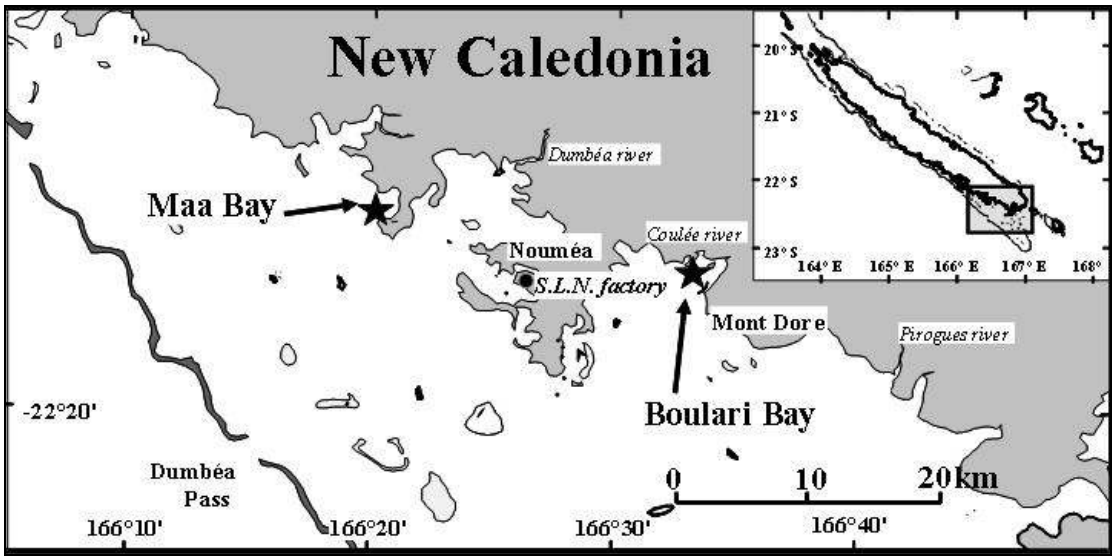
Therefore the aim of this study was to determine the bioconcentration and depuration capacity of *L. variegata* for 9 selected elements (Ag, As, Cd, Co, Cr Cu, Mn, Ni and Zn) in field conditions using cross-transplantation experiments in the New Caledonia lagoon. These experiments were carried out in order to determine whether transplanted algae could bioconcentrate and depurate metals up to the values found in the resident populations of the transplantation site and the time necessary for them to reach concentrations representative of these latter populations.

II. MATERIALS AND METHODS

II.1. SELECTION OF TRANSPLANTATION LOCATIONS

Recent studies provided data on element concentrations in the alga *Lobophora variegata* collected from different parts of the lagoon of New Caledonia (Breau 2003; Hédouin et al. submitted-a). These data were used for selecting the “reference” site and the “contaminated” site where the cross transplantation experiments were carried out. Maa Bay was identified as the reference site (low element concentrations measured in algae as well as in sediments) and Boulari Bay as the contaminated site (very high element concentrations measured in algae and sediments, due to terrigeneous metal inputs from the conjunction of natural and mining-induced soil erosion) (Fig. 1).

Figure 1. Location of the two transplanted sites: Maa Bay and Boulari Bay, New Caledonia.



II.2. EXPERIMENTAL DESIGN

Fifty specimens of *L. variegata* were collected in February 2005 in Maa Bay. Ten individuals were analysed for their contents in Ag, As, Cd, Co, Cr Cu, Mn, Ni and Zn (see procedure below in section II.3.) in order to establish the baseline concentrations at the beginning of the experiment. The 40 remaining algae were transplanted for 103 d in the contaminated site (Boulari Bay). A mirroring transplantation was undertaken with algae collected in Boulari Bay and transplanted in Maa Bay.

In addition to the 10 algae sampled at time 0 in each site, 5 individuals of the resident population of *L. variegata* and 5 transplanted organisms (in both reference and contaminated sites) were collected after 6, 16, 23, 29, 41, 55, 77 and 103 d in order to compare the change and variability of element concentrations in transplanted and resident populations. At time 0, all organisms, including the control resident specimens, were placed in plastic cages placed between 4 and 5 m depth and made of 1-cm meshed plastic net to ensure free water circulation between the inside of the cage and the surrounding seawater. Transplanted and control algae were collected by SCUBA diving, transported to the laboratory in clean, acid-washed PET bags, and processed for element analysed the same day (typically within 4 to 5 hrs).

II.3. SAMPLE PREPARATION AND ANALYSES

Back to the laboratory, the algae were primarily cleansed of their epiphytes by gentle scrubbing and of attached sediment grains by several rinsings in seawater from the sites of collection. The algae were then weighed (wet wt), dried at 60°C until constant weight, and weighed again (dry wt) before being stored in acid-washed hermetically sealed PET containers until further analysis.

Biological samples (200-300 mg dry wt) were digested using 8 ml of a 3: 1 (v: v) 65 % HNO_3 -30 % HCl mixture (Merck, suprapur quality) and 0.5 ml of 40 % HF (Merck, suprapur quality). Acidic digestion was first carried out overnight at room temperature then mineralized using a MARS V microwave (30-min long linear increase up to 115°C followed by 15 min at 115°C). Each sample volume was then adjusted to 50 ml with milli-Q water.

Elements were analysed using a Varian Vista-Pro ICP-OES (As, Cr, Cu, Mn, Ni, and Zn) or a Varian ICP-MS Ultra Mass 700 (Ag, Cd and Co). Three control samples (two certified reference materials –CRM– and one blank), treated and analysed in the same way as the samples, were included in each analytical batch. CRM were dogfish liver DOLT-3 (NRCC) and lobster hepatopancreas TORT-2 (NRCC). The results from CRM analysis indicated a recovery ranging from 81 % (Ni) to 113 % (Zn) (Table 1). The detection limits were 31 (As), 1.3 (Cr), 3.8 (Cu), 0.15 (Mn), 1.1 (Ni) and 2.4 (Zn) $\mu g\ g^{-1}$ dry wt for ICP-OES and 0.1 (Ag), 0.15 (Cd) and 0.1 (Co) $\mu g\ g^{-1}$ dry wt for ICP-MS.

Table 1. ICP-OES and ICP-MS analyses of certified reference materials: certified and measured values (mean ± SD; µg g^{-1} dry wt; n = 4).

Elements	Method	TORT-2			DOLT-3		
		Measured	Certified	% Recovery	Measured	Certified	% Recovery
		Mean ± SD	*Mean ± SD*		*Mean ± SD*	*Mean ± SD*	
Ag	**ICP-MS**				1.07 ±	1.20 ± 0.07	89.3
As	**ICP-OES**	22.28 ± 2.22	21.60 ± 1.80	103.2	9.45 ± 0.97	10.20 ± 0.50	92.7
Cd	**ICP-MS**	26.42 ± 3.75	26.70 ± 0.60	99.0	17.01 ± 3.12	19.40 ± 0.60	87.7
Co	**ICP-MS**	0.52 ± 0.09	0.51 ± 0.09	101.5			
Cr	**ICP-OES**	0.66 ± 0.19	0.77 ± 0.15	85.3			
Cu	**ICP-OES**	98.40 ± 11.17	106.00 ± 10.00	92.8	31.23 ± 2.40	31.20 ± 1.00	100.1
Mn	**ICP-OES**	12.46 ± 1.19	13.60 ± 1.20	91.6			
Ni	**ICP-OES**	2.02 ± 0.35	2.50 ± 0.19	80.9	3.05 ± 0.76	2.72 ± 0.35	112.1
Zn	**ICP-OES**	187.60 ± 19.63	180.00 ± 6.00	104.2	97.67 ± 6.97	86.60 ± 2.40	112.8

II.4. STATISTICAL ANALYSES

Uptake and depuration kinetics of the elements were determined using simple linear regression equations:

uptake kinetics: $C_t = C_0 + k_u\, t$

and

loss kinetics: $C_t = C_0 - k_e\, t$

where C_t and C_0 are the element concentration (µg g^{-1} dry wt) in algae at time t (d) and 0, respectively, and k is the uptake (k_u) or loss (k_e) rate constant (µg g^{-1} dry wt d^{-1}). Constants of the equation and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica® 5.2.1 software.

At the starting day (t_0) of the transplantations, element concentrations in algae from reference site were compared to those from contaminated stations using one-way analysis of variance (ANOVA) followed by the multiple comparison test of Tukey (Zar 1996). When transplanted

algae displayed a significant linear increase in element concentrations with time, element concentrations in transplanted organisms at the end of transplantation period were compared to those of resident algae from the transplanted stations (1-way ANOVA). In addition, if element concentrations in resident algae showed a significant increase/decrease with time, the slope of the regression (k_u or k_e) was statistically compared with the slope of the regression for transplanted algae (Zar 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

III. RESULTS

III.1. STARTING DAY OF TRANSPLANTATION

On t_0, the concentrations of As, Ag, Co, Cr, Mn and Ni were significantly higher in algae from Boulari Bay than in those from Maa Bay ($p_{Tukey} < 0.0006$ for Ag, As, Co, < 0.008 for Mn and Ni, < 0.02 for Cr), whereas no significant difference was found for Cd, Cu and Zn concentrations between the two sites.

III.2. TRANSPLANTATION FROM MAA BAY TO BOULARI BAY

Element concentrations in the resident *L. variegata* population from Maa Bay did not varied significantly during the experiment for all elements, indicating that any variations in concentrations in algae transplanted to Boulari Bay were actually related to changes in environmental conditions.

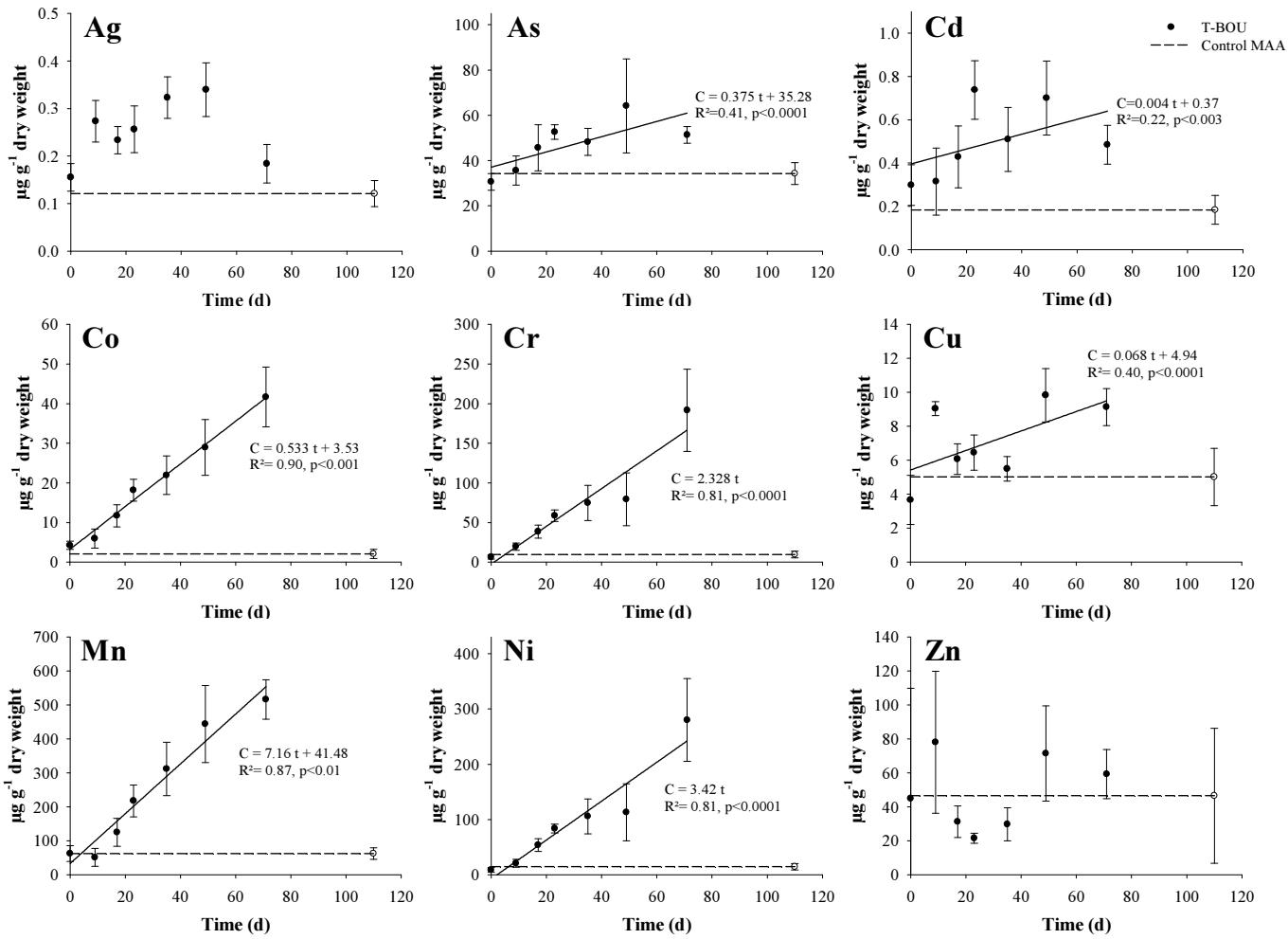
When algae were transplanted from the reference site (Maa Bay) to the contaminated site (Boulari Bay), their concentrations in As, Cd, Co, Cr, Cu, Mn and Ni increased linearly over the observation period ($R^2 = 0.81 – 0.90$ for Co, Cr, Mn and Ni, and $R^2 = 0.22$ - 0.41 for As, Cd and Cu) (Fig. 2). Uptake rate of Cr, Mn and Ni was higher by one to three orders of magnitude than for the other elements. The concentrations of Cr, Mn and Ni increased respectively from 6.5, 63 and 9.0 $\mu g\ g^{-1}$ dry wt at the beginning of the experiment up to 192, 516 and 280 $\mu g\ g^{-1}$ dry wt after 71 d of transplantation.

For Ag and Zn, no significant increase in concentration was found during the transplantation period ($p_{regression\ slope} = 0.06$ for Ag and 0.5 for Zn).

The concentrations of As, Cd, Co, Cr, Cu, Mn and Ni in algae at the end of the transplantation period in Boulari Bay were compared to those of resident algae from Boulari Bay. Results showed that the concentrations of all these elements but As were significantly higher in transplanted algae than in the resident algal population from Boulari Bay (from 1.6 to 2.9 fold; p_{Tukey} always < 0.003). No significant difference was observed for As.

Figure 2. Element concentrations (mean ± SD; µg g^{-1} dry wt; n = 5) in *Lobophora variegata* transplanted from Maa Bay (reference site) to Boulari Bay (contaminated site).

Solid lines indicate significant variation in element concentrations in transplanted algae; broken lines indicate mean element concentrations in resident algae from Maa Bay (n = 30).

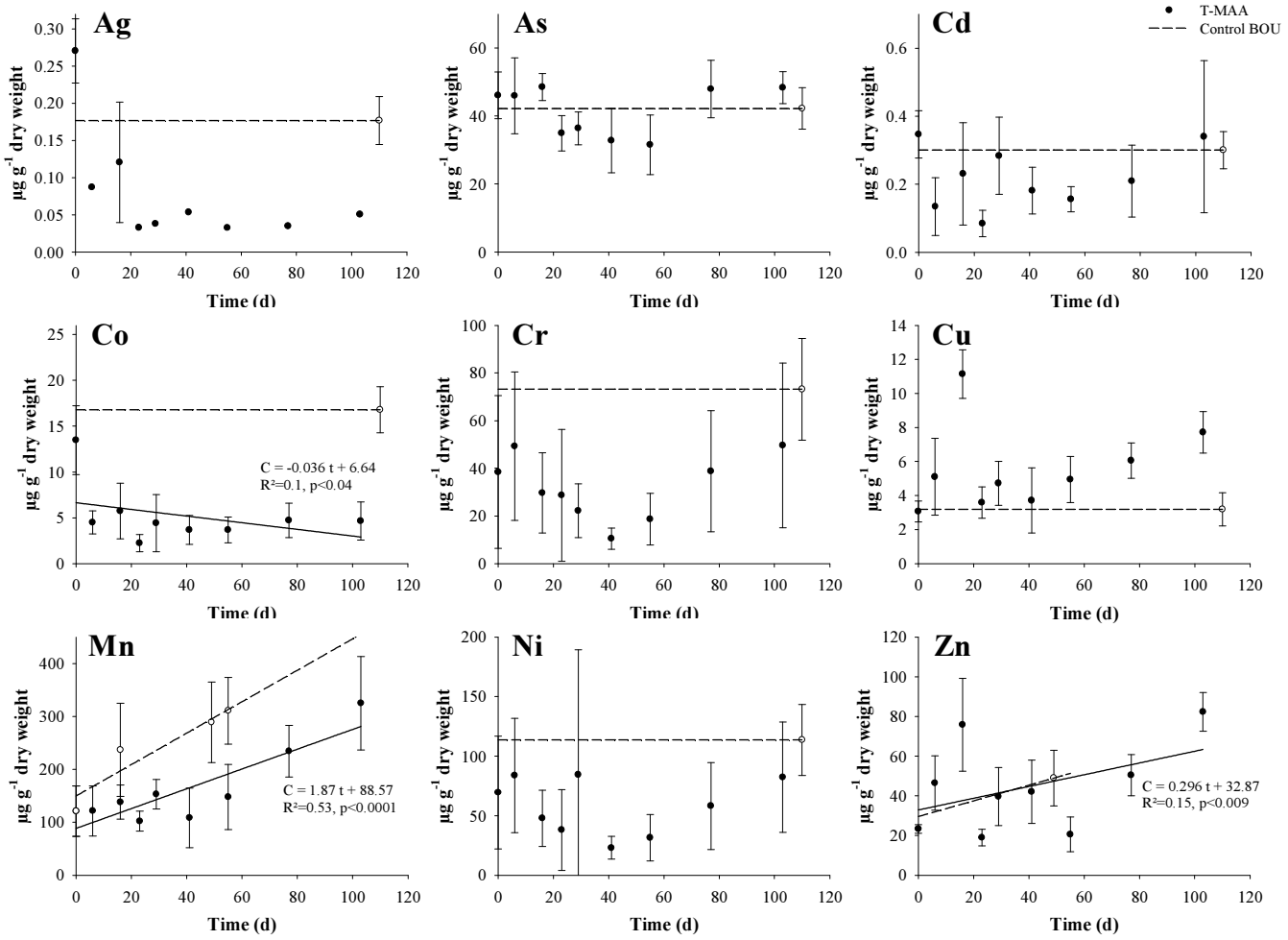


III.3. TRANSPLANTATION FROM BOULARI BAY TO MAA BAY

During the transplantation experiment from the contaminated to the reference site, Co concentrations decreased linearly (k_e: 0.036 µg g^{-1} dry wt d^{-1}; R^2 = 0.1) whereas Mn and Zn concentrations increased linearly (k_u = 1.87 and 0.30 µg g^{-1} dry wt d^{-1}, R^2 = 0.53 and 0.15, respectively) (Fig. 3). With the exception of day 16, Ag concentrations in algae was below the detection limit (0.1 µg g^{-1} dry wt), precluding any regression fit calculation. No significant linear regression could be calculated for all the other elements.

Figure 3. Element concentrations (mean ± SD; µg g^{-1} dry wt; n = 5) in *Lobophora variegata* transplanted from Boulari Bay (contaminated site) to Maa Bay (reference site).

Solid lines indicate significant variation in element concentrations in transplanted algae; broken lines indicate mean element concentrations in resident algae from Boulari Bay (n = 30).



No significant variation in element concentrations was observed in the *L. variegata* resident population in Boulari Bay, except for Mn and Zn. For these two latter metals a significant increase of concentration was found. The corresponding estimated uptake rate constants (k_u = 3.18 and 0.39 μg g^{-1} dry wt d^{-1} for Mn and Zn respectively, $p < 0.04$) were found to be not significantly different from those calculated in algae originating from Boulari Bay and transplanted in Maa Bay.

At the end of the experiment, Co and Mn concentrations were significantly higher (p_{Tukey} = 0.03 and 0.0002, respectively) in algae transplanted in Maa Bay than in resident algae from Maa Bay (1.9 fold higher for Co and 5.2 for Mn), whereas no significant difference was found for Zn.

IV. DISCUSSION

When transplanted in the contaminated site, the tropical brown alga *L. variegata* significantly took up As, Cd, Co, Cr, Cu, Mn and Ni, which demonstrated that a significant part of the elements was occurring as bioavailable forms and confirmed that the target algal species has a strong potential to accumulate these contaminants. In a comparable transplantation experiment in Sepatiba Bay, Brazil (1 month duration), Amado Filho et al. (1999) observed that the brown alga *Padina gymnospora* rapidly accumulated Cd and Zn.

Our results showed that when transplanted, *L. variegata* is able to rapidly respond to an increase in ambient contaminant concentration. Individuals transplanted from the reference site (Maa Bay) to the contaminated site (Boulari Bay) reached concentrations in Co, Cr, Mn and Ni similar to those measured in resident algae from Boulari Bay after about one month. Surprisingly, beyond that period, concentrations in Cd, Co, Cr, Cu, Mn and Ni continued to increase linearly to reach values significantly higher than those measured in the resident population. This pattern indicates that when subject to significant contaminant levels for a long time, resident algae could have developed adaptative response(s) to handle high contamination pressure by e.g. regulating intake or loss rate of contaminants, through either physiologic acclimation or genetic adaptation (Klerks & Weis 1987; Ma et al. 2000). This is further supported by laboratory experiments, which demonstrated that during short-term exposures (14-d), viz. without any possible adaptation, uptake of Cd, Co, Cr, Mn, Ni and Zn in *L. variegata* from a single population was linear during the experiment duration, reaching

tissue concentrations directly proportional to the concentrations of the metals in seawater over 2 to 3 orders of magnitude (Metian et al. submitted).

The strong bioaccumulation potential for As, Cd, Co, Cr, Cu, Mn and Ni and the ability of the alga to provide quantitative information on contaminant levels in the surrounding environment as indicated by both laboratory and field studies converge in demonstrating the relevance of *L. variegata* as a bioindicator species to be used in the New Caledonian lagoon. For biomonitoring applications, contaminant levels in the marine environment could be monitored by using resident algae since they are able to discriminate stations according to their contamination status (Hédouin et al. submitted-a). However, the present study indicates that using transplanted algae could allow measuring contaminant levels that would be higher than those found in resident populations. This suggests that whereas a proportional relationship between contaminant concentration in transplanted algae and those in the environment exists (as has been shown from the laboratories studies, Metian et al. submitted), a breakdown may occur in this relationship for resident algae, due to probable adaptation to their environment. Therefore, the use of transplanted algae would allow by-passing these adaptation processes in resident algal population and would thus be a more sensitive and discriminating tool to assess the level of contamination occurring in different stations.

While *L. variegata* shows a rapid and efficient response to contaminated environment, things appeared strongly different when algae were transplanted from the contaminated site to the reference site. Indeed, except for Co for which some significant depuration occurred, the concentrations in the other elements were similar after 3 months of transplantation than at the starting time. This observation is somewhat contrasting with previous results from laboratory radiotracer experiments which estimated biological half-lives ($T_{b½}$) ranging from 37 d to an infinite time for Cd, Co, Cr, Mn, and Zn and 6-8 d for Ni.

Phaeophyceae are well known to strongly bind metal ions (see e.g., Bryan 1984); their metal uptake depends on adsorption (biosorption), where elements are bound on the cell walls, and/or on absorption, where elements enter within the cells and bind strongly with macromolecules such as polyphenols, phytochelatins and metallothioneins (e.g. Ragan et al. 1979; Morris et al. 1999; Cobbett & Goldsbrough 2002). Polyphenols being present in very large proportion in *L. variegata* (8.33 % to 13.39 % dry wt) (Targett et al. 1992b), it is very likely that absorption would be a much more significant process than adsorption, especially for metals that showed very long retention times in algae.

Beside being responsible of the high bioconcentration efficiency of the algae, the high content in these metal-binding macromolecules could also explain the virtual lack of contaminant depuration in algae transplanted from the contaminated to the reference site during the observation time. Indeed, past exposure history may influence further contaminant elimination, as it has been reported for example in oysters (Wallner-Kersanach et al. 2000). As hypothesized above, if algae from the contaminated area have developed efficient detoxification strategies based on metal sequestration (e.g. via their important content in phytochelatins), most of the tissue-associated metal pool would be strongly bound to intracellular components which would logically result in an inertial metal retention, even when transplanted in a non-contaminated area. Nevertheless, in order to better understand the elimination and detoxification processes of metals in *L. variegata*, further *in situ* experimental studies should be conducted based on longer term elimination experiments and considering algae with different exposure histories. This could be done carrying out field depuration experiments with (1) algae coming from a contaminated sites and (2) algae collected from a reference site, then transplanted for a few months in a contaminated site prior being replaced in the reference site to follow metal depuration.

In the particular case of Mn and Zn, an increase in concentrations was observed in algae from the contaminated site (Boulari Bay) transplanted to the reference site (Maa Bay). However, an identical increase in concentrations was measured in the resident algae from Boulari Bay, which strongly suggests that this observation was due to specific physiological parameters rather than to an actual uptake of Mn and Zn due to possible higher contamination levels in Mn and Zn in the reference site. This is further supported by the fact that in the uptake experiment, algae from Maa Bay transplanted to Boulari Bay actually took up Mn very efficiently (tissue concentrations increased by two orders of magnitude) whereas no difference in Zn levels was found between the two sites, thus indicating that Maa Bay is actually similarly (Zn) or much less (Mn) contaminated than Boulari Bay.

V. CONCLUSION

The cross-transplantation demonstrated that the alga *L. variegata* is a powerful bioindicator of metal contamination in the New Caledonia lagoon as it displays high bioconcentration capacities, especially for Co, Cr, Mn and Ni. Although another recent study showed that concentrations measured in resident *L. variegata* populations (passive biomonitoring) allow

the discrimination of the degree of contamination of their environment (Hédouin et al. submitted-a), the present *in situ* transplantation experiments provided essential data on the particular relevance of this species for active biomonitoring. Hence this would allow for extending monitoring studies to areas of the New Caledonia lagoon where the species *L. variegata* is not naturally present. Moreover, due to its wide distribution in tropical areas (Targett et al. 1992b), the information gathered on the brown alga *L. variegata* certainly has great potential in its application as a metal contamination biomonitoring tool in numerous other tropical ecosystems, where information on adequate bioindicators are strongly lacking.

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CHAPITRE 10

Expériences de transplantation pour la validation de deux espèces marines tropicales comme bioindicateurs de la contamination minière du lagon de Nouvelle-Calédonie

L'huître *Isognomon isognomon* et le clam *Gafrarium tumidum* ont été précédemment proposés en tant qu'espèces bioindicatrices potentielles pour la surveillance de la contamination métallique due aux activités minières dans le lagon de Nouvelle-Calédonie. L'objectif de cette étude était de déterminer les capacités de bioaccumulation et de rétention d'une série de contaminants clés (Ag, As, Cd, Co, Cr, Cu, Mn, Ni et Zn) dans les deux espèces lors d'expériences de transplantation. Les cinétiques d'accumulation et d'élimination déterminées *in situ* ont montré que les concentrations en Co, Ni et Zn dans les clams transplantés, et en Cr et Cu dans les huîtres transplantées atteignent celles des populations résidentes, alors que pour d'autres éléments, un temps plus long que la durée de l'expérience sera nécessaire aux organismes transplantés pour atteindre les niveaux des populations résidentes (par exemple, jusqu'à 35 mois pour le Cd). De plus, la transplantation de clams et d'huîtres collectés dans des sites propres dans deux stations fortement contaminées (GR1 et GR2) montrent qu'après 35 jours de transplantation, bien que les deux espèces accumulent de manière significative le Co, Cr et Ni, le clam possède une capacité de bioaccumulation plus efficace que l'huître. Quand des organismes provenant de sites contaminés sont transplantés dans des sites propres, les contaminants sont très peu éliminés des tissus des organismes, à l'exception de l'Ag, du Co et du Ni dans les huîtres.

Ainsi, les résultats de ces deux expériences suggèrent que le clam est un bioindicateur de la contamination minière plus intéressant que l'huître de part ses capacités de bioaccumulation plus importante.

Transplantation experiments for the validation of two tropical marine bivalves as bioindicators of mining contamination in the New Caledonian lagoon

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To be submitted

ABSTRACT. The oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* have been previously suggested as potential bioindicator species for monitoring mining-originating metal contamination in the New Caledonia lagoon. The objective of the present study was to determine the bioaccumulation and retention capacity of a series of key local contaminants (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn) in the two species during transplantation experiments. Uptake and depuration kinetics determined in the field indicated that whereas for Co, Ni and Zn in clams, and Cr and Cu in oysters, concentrations in transplanted bivalves reached those of resident organisms, for other elements, it would required a time longer than the duration of the experiment for transplanted bivalves to reach levels of residents populations (e.g. up to 35 months for Cd). Moreover, when clams and oysters from clean sites were transplanted into a two highly contaminated station (GR1 and GR2), after 35 d of transplantation, although both species exhibited significant increased in Co, Cr, and Ni concentrations, the clam displays a higher bioaccumulation capacity than the oyster. When contaminated organisms were transplanted to clean environment, no major depuration of metals occurred in the both bivalves, except for Ag, Co and Ni in oysters.

Therefore, results from both experiments suggest that *G. tumidum* is a more interesting bioindicator of mining contamination than *I. isognomon,* since it is able to bioaccumulate contaminant with higher efficiency.

Keywords: *Isognomon isognomon, Gafrarium tumidum,* Bioaccumulation, Biomonitoring

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I. INTRODUCTION

New Caledonia is a small South Pacific island whose main economic resources are derived from nickel exploitation. Local mining activities result in important anthropogenic inputs of metals into the SW lagoon and thereby constitute a threat to the local coastal marine ecosystems (e.g., Bird et al. 1984; Ambatsian et al. 1997). However, very little information is available regarding levels of metal contamination and possible impact on the local marine ecosystems (Monniot et al. 1994; Breau 2003). Monitoring of environmental contamination originating from mining activities in the lagoon is therefore a matter of concern.

Among the common approaches used to study environmental contamination, the use of bivalve molluscs as bioindicator species proved to be a valuable and informative approach (e.g. Mussel Watch, Goldberg et al. 1983). This approach has been particularly developed in temperate areas whereas, in sub-tropical and tropical areas, the scarcity of available information makes difficult the identification of species that could be used as suitable bioindicators (e.g., Phillips 1991). However, a recent study has screened metal concentrations in a variety of marine organisms from several parts of the SW lagoon of New Caledonia (Breau 2003) and has identified the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* as potential bioindicator for surveying local metal contamination. More recently, it has also been shown experimentally that these two species bioconcentrate and retain efficiently numerous elements when exposed via seawater or the food (Hédouin et al. submitted-d; Hédouin et al. to be submitted-b). More importantly, both species were shown to concentrate from seawater and absorb from food As, Cd, Co, Cr, Mn, Ni, Zn in direct proportion to their concentrations in seawater and the food, respectively (Hédouin et al. submitted-b; Hédouin et al. submitted-c).

Although the former experiments were carried out under controlled conditions simulating as closely as possible the natural ones, laboratory cannot reproduce exactly the field. In this respect *in situ* experiments offer a more ecologically realistic approach, since they encompass all the factors that actually occur in the field and may possibly interfere with or influence bioaccumulation processes. Hence, transplantation experiments have been widely used to assess metal bioaccumulation capacities of marine organisms in the field and/or to validate their use as bioindicator species in temperate areas (e.g. Cain & Luoma 1985; Riget et al. 1997; Abbe et al. 2000). In tropical environments, only four similar field studies have been

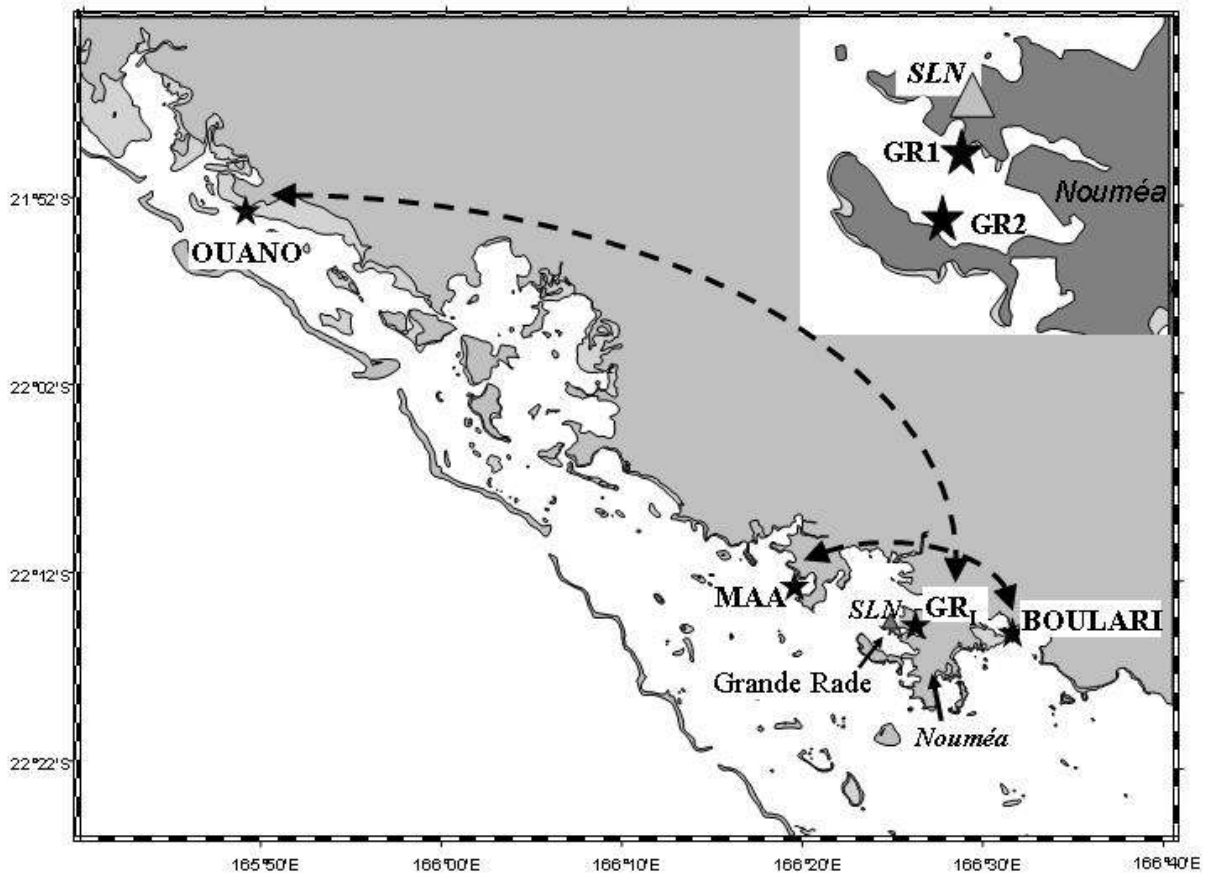
reported (Lim et al. 1995; Wallner-Kersanach et al. 2000; Olivier et al. 2002; do Amaral et al. 2005).

The aim of the present study was to determine the relevance of using the clam *Gafrarium tumidum* and the oyster *Isognomon isognomon* as bioindicator species of metal contamination. The ability of both species to bioaccumulate and depurate 9 selected elements (Ag, As, Cd, Co, Cr Cu, Mn, Ni and Zn) under natural conditions has been determined during field transplantation experiments (active biomonitoring), as well as their ability to inform about the contamination status of their surrounding environment. A specific approach was also carried out to determine the sample size required to allow for discriminating among realistic field contamination levels.

II. MATERIALS AND METHODS

Two transplantation experiments have been performed in New Caledonia, between March and June 2005, using the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum*. Recent studies have measured element concentrations in *I. isognomon* and *G. tumidum* from different sampling stations in New Caledonia (Breau 2003; Hédouin et al. submitted-a). Based on these results, sampling stations have been selected according to their contamination status. Maa Bay for oysters and Ouano Beach (intertidal station) for clams have been identified as clean stations for oysters and clams, respectively, with low element concentrations in bivalve tissues and sediments for all elements except As for oysters and clams, respectively. In contrast, Boulari Bay (for oysters) and Grande Rade -GR_I- (for clams) have been identified as highly contaminated stations (Fig. 1).

Figure 1. Map showing the selecting stations for transplantation experiments.



II.1. EXPERIMENTAL DESIGN

Since body size is well-known to affect metal concentrations in organisms (Boyden 1977); only samples with shell width longer than 35 mm for *G. tumidum* (Hédouin et al. in press) and shell length longer than 70 mm for *I. isognomon* (Metian 2003) were considered in order to minimize size-related variability.

II.1.1. Reciprocal transplantations

Eighty clams *G. tumidum* and 80 oysters *I. isognomon* were collected from the two selected clean stations, Ouano Beach and Maa Bay, respectively. A sub-sample of 10 organisms from each station was used for determination of baseline concentrations of the 9 selected elements (Ag, As, Cd, Co, Cr Cu, Mn, Ni and Zn) at the beginning of the experiment. The remaining clams and oysters (n = 70 per species) were transplanted for 100 d to the highly contaminated

stations, Grande Rade, (GR_I) and Boulari Bay, respectively. The reciprocal transplantation was undertaken with another batch of 80 clams and 80 oysters collected in Grande Rade (GR_I, intertidal station) and Boulari Bay and transplanted to the clean stations, Ouano Beach and Maa Bay, respectively.

Organisms (transplanted and control resident individuals) were placed at each station in plastic meshed cages, allowing free exchanges between the inside and the surrounding water. The plastic cages containing the oysters were placed at 4-5 m depth; those of clams were placed in an intertidal position and inserted within the sediments in order to reproduce as well as possible the living condition of the clams. In order to monitor possible natural variation in element concentrations at the different stations, resident organisms (n = 5 per species) and superficial sediments (top 5-cm layer) were sampled simultaneously with the transplanted organisms (n = 7) from clean and contaminated stations at different times. Clams were collected by handpicking, whereas the oysters were collected by SCUBA diving.

II.1.2. Transplantation in Grande Rade

Grande Rade is locally influenced by anthropogenic inputs from the metallurgic factory 'Société Le Nickel' (SLN). Two stations (GR1 and GR2) were chosen in Grande Rade for the second experiment on the assumption that they displayed different contamination status. GR1 station is supposed being a highly polluted site due to its proximity to the unload decks of the SLN, whereas the second transplanted station GR2 on the opposite side of the Rade, in front of the SLN factory, is supposed to be less contaminated than GR1 (Fig. 1).

The bivalves *I. isognomon* and *G. tumidum* (n = 80 per species) were collected from the clean stations Maa Bay and Ouano Beach, respectively. Twenty organisms were used for element analyses in order to establish the baseline concentrations of elements at the day 0 of transplantation; whereas the remaining organisms (n = 60 per species) were transplanted for 69 d to the two transplanted stations in Grande Rade (GR1 and GR2), in plastic caged immerged at 5 m depth for both clams and oysters. Samples (n = 30) of transplanted organisms in GR1 and GR2, and resident organisms (n = 20) from the clean stations (Maa Bay for oysters and Ouano Beach for clams) were collected by SCUBA diving after 35 and 69 d. Sediment samples were collected simultaneously with organisms in the clean and transplanted sites.

II.2. SAMPLING PREPARATION AND ANALYSES

Back to the laboratory, the bivalves were kept for 24 h in 30 l seawater from the same sampling station to allow for depuration of gut contents and of particulate material present in the mantle cavity. Soft tissues were removed from the shells and were weighed (wet wt), dried at 60°C until constant weight, and weighed again (dry wt). They were then stored in acid-washed, hermetically-sealed plastic containers until analysis.

Sediments were stored in acid-washed and hermetically-sealed plastic bags until return to the laboratory; then dried at 60°C for 5 d. In order to eliminate heterogeneous materials (e.g. stones, fragment of corals), sediments were sieved (1-mm mesh size) prior to analysis.

Aliquots of the biological samples (300 to 500 mg) and aliquots of sediment samples (300 mg) were digested using a 3: 1 (v: v) nitric-hydrochloric acid mixture (65 % suprapur HNO_3 and 30 % suprapur HCl, Merck). Acidic digestion of the samples was carried out overnight at room temperature. Samples were then mineralized using a MARS V microwave (30 min with constantly increasing temperature up to 100°C for sediment and 115°C for biological material, then 15 min at these maximal temperatures). Each sample was then diluted to 30 or 50 ml with milli-Q water according to the amount of sample digested.

Elements were analysed using a Varian Vista-Pro ICP-OES (As, Cr, Cu, Mn, Ni, and Zn) and a Varian ICP-MS Ultra Mass 700 (Ag, Cd and Co). Three control samples (two Certified Reference Material - CRM - and one blank) treated and analysed in the same way as the samples, were included in each analytical batch. CRM were dogfish liver DOLT-3 (NRCC) and lobster hepatopancreas TORT-2 (NRCC). The results for CRM indicated recoveries of the elements ranging from 81 % (Ni) to 113 % (Zn) (Table 1). The detection limits were 31.0 (As), 1.3 (Cr), 3.8 (Cu), 0.15 (Mn), 1.1 (Ni) and 2.4 (Zn) $\mu g\ g^{-1}$ dry wt for ICP-OES and 0.1 (Ag), 0.15 (Cd) and 0.1 (Co) $\mu g\ g^{-1}$ dry wt for ICP-MS. All element concentrations are given on a dry weight basis ($\mu g\ g^{-1}$ dry wt).

Table 1. ICP-OES and ICP-MS Analysis of certified reference materials: certified values and found values (mean ±SD µg g^{-1} dry weight) (n.c. : no certified value).

		TORT-2					DOLT-3				
		Found		*Certified*		*% Recovery*	*Found*		*Certified*		*% Recovery*
		Mean	*SD*	*Mean*	*SD*		*Mean*	*SD*	*Mean*	*SD*	
Ag	ICP-MS						1.07	0.09	1.20	0.07	89.3
As	ICP-OES	22.28	2.22	21.60	1.80	103.2	9.45	0.97	10.20	0.50	92.7
Cd	ICP-MS	26.42	3.75	26.70	0.60	99.0	17.01	3.12	19.40	0.60	87.7
Co	ICP-MS	0.52	0.09	0.51	0.09	101.5					
Cr	ICP-OES	0.66	0.19	0.77	0.15	85.3					
Cu	ICP-OES	98.40	11.17	106.00	10.00	92.8	31.23	2.40	31.20	1.00	100.1
Mn	ICP-OES	12.46	1.19	13.60	1.20	91.6					
Ni	ICP-OES	2.02	0.35	2.50	0.19	80.9	3.05	0.76	2.72	0.35	112.1
Zn	ICP-OES	187.60	19.63	180.00	6.00	104.2	97.67	6.97	86.60	2.40	112.8

II.3. DATA TREATMENT AND STATISTICAL ANALYSES

Elements uptake kinetics were described using either a simple linear regression model (eq.1) or, a saturation exponential model (eq.2):

$C_t = C_0 + k_u\, t$ (eq.1)

$C_t = C_0 + C_1\,(1 - e^{-k_e\, t})$ (eq.2)

where C_t and C_0 are the element concentrations in organisms at time t (d) and 0; $C_1 + C_0$ is the concentrations at steady state (C_{ss}); k_u (= $C_1\, k_e$) is the uptake rate constant (µg g^{-1} d^{-1}) and k_e is the depuration rate constant (d^{-1}) (Whicker & Schultz 1982).

Loss kinetics of elements were described by either a simple linear regression model (eq.3) or a single-component exponential equation (eq.4):

$C_t = C_0 - k_e\, t$ (eq.3)

$C_t = C_0\, e^{-k_e\, t} + A$ (eq.4)

where C_t and C_0 are the element concentration in organisms at time t (d) and 0; k_e the depuration rate constant (d^{-1}) and A is a constant.

Model constants and their statistics of uptake and loss kinetics were estimated by iterative adjustment of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica software 5.2.1.

Element concentrations of sediments and control organisms were plotted against time and were fitted using simple linear regression. Statistical analyses of the data were performed using 1-way analysis of variance (ANOVA) followed by the multiple comparison test of Tukey (Zar 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

A test of Power was performed using the whole set of data in order to assess the minimal sample size of organisms (clams and oysters) required to detect realistic (field-observed) differences in element concentration with statistical significance ($p < 0.05$) (Zar 1996).

III. RESULTS

III.1. ACTIVE BIOMONITORING USING RECIPROCAL TRANSPLANTATIONS

III.1.1. Sediment

In sediment collected in the contaminated station Grande Rade (GR_I), concentrations of all elements were significantly higher (p_{Tukey} always ≤ 0.0002) than those from the clean station (Ouano Beach) (Table 2).

Concentrations of As, Co, Cr, Mn and Ni in sediments collected from Boulari Bay were significantly higher (p_{Tukey} always ≤ 0.0008) than those collected from Maa Bay, whereas concentrations of Cu and Zn were significantly higher (p_{Tukey} always ≤ 0.0002) in Maa Bay compared to Boulari Bay (Table 2). No significant difference was observed between Cd concentrations in sediments from Boulari Bay and Maa Bay.

Element concentrations in sediment collected from the four stations showed no major variation with time.

Table 2. Element concentrations (mean ± SD; µg g^{-1} dry wt, n = 3) in sediment collected in six sampling locations.

	OUANO	MAA	BOULARI	GR_I	GR1	GR2
Ag	*	*	**0.1**±0.09	**0.4***±0.1	**0.2***±0.3	*
As	**3.1*** ± 1.2	**6.4***±0.3	**16.7***±1.3	**8.0***±1.2	**7.0***±5.9	**15.4**±0.5*
Cd	**0.4** ±0.2	**1.0**±0.2	**1.1**±0.3	**2.5**±0.2	**3.7**±1.2	**0.8**±0.1
Co	**0.8**±0.4	**4.4**±2.3	**15.4**±11.1	**49.2**±5.2	**366**±145	**6.1**±0.9
Cr	**7.8**±2.4	**46.9**±4.0	**71.5**±10.2	**308.8**±39.0	**1293**±410	**24.6**±2.9
Cu	**1.4***±0.7	**7.0**±0.5	**0.9***±0.1	**27.0**±3.6	**9.6**±3.3	**2.8***±0.4
Mn	**44.7**±14.9	**133.6**±6.7	**545.0**±53.0	**303.7**±14.8	**1596**±603	**76.7**±8.1
Ni	**5.6**±3.0	**69.2**±5.6	**101.1**±12.9	**847.8**±77.7	**10454**±3310	**66.4**±15.8
Zn	**3.5**±2.0	**16.3**±1.3	**7.1**±1.6	**147.5**±11.0	**73.3**±22.7	**12.8**±1.8

* Inferior to detection limit

III.1.2. Clams *G. tumidum*

At the beginning of the transplantation experiment (day 0), concentrations of all elements in clams from Ouano Beach were significantly lower (p_{Tukey} always ≤ 0.001, except for Mn and Zn, p ≤ 0.02) than those from Grande Rade (GR_I), except for As, for which the highest concentration (p_{Tukey} = 0.0003) was measured in clams from Ouano Beach, and for Cd, for which no significant difference was found between the two stations.

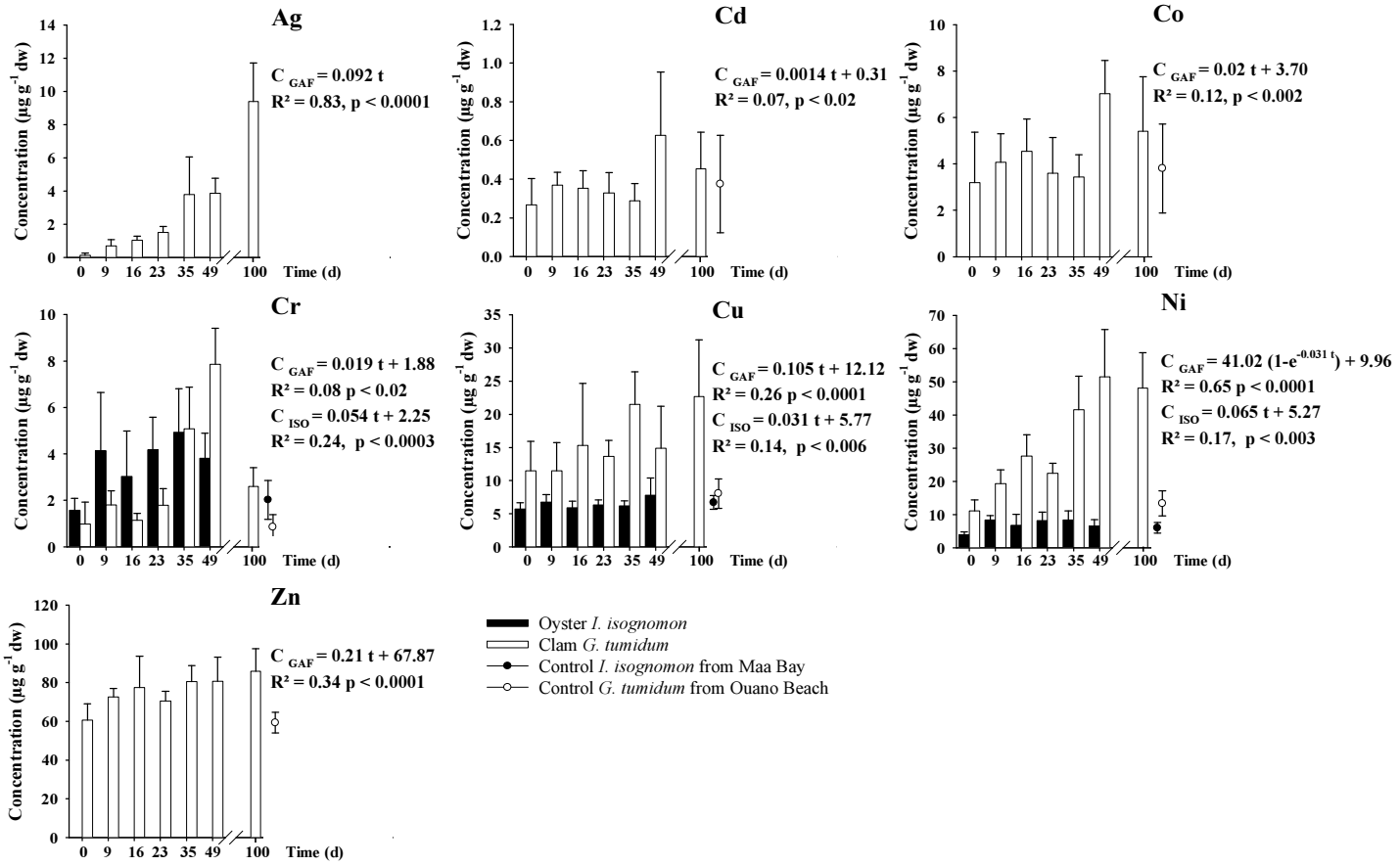
Control resident *G. tumidum* in Ouano Beach and Grande Rade showed no significant variation for any elements along the duration of the experiments.

In clams transplanted to the contaminated station (GR_I), the concentrations of Ag, Cd, Co, Cr, Cu and Zn displayed a significant linear increase (R^2: 0.26 - 0.83, except for Cd, Co, Cr; R^2 < 0.12) (Fig. 2). The uptake kinetics of Ni in clam soft tissues was best fitted by an exponential model (R^2 = 0.65, p < 0.0001) for which the estimated uptake rate constant, k_u, was 1.27 µg g^{-1} d^{-1}. The uptake rate of Cu, Ni and Zn was higher by one order of magnitude compared to the one of the other elements.

When clams from GR_I were transplanted to the clean station, Ouano Beach, Ag and As concentrations displayed a significant linear increase (k_e: -0.08 and -0.54; R^2: 0.17 and 0.56, respectively) (Fig. 3). For the other elements, no significant depuration has been observed.

Figure 2. Element concentrations (mean ± SD; µg g-1 dry wt; n = 7 for transplanted organisms and n = 5 for control organisms) in clams *Gafrarium tumidum* and oysters *Isognomon isognomon* transplanted from clean stations, Ouano Beach and Maa Bay, to the contaminated stations, Grande Rade and Boulari Bay, respectively.

(only data shown a significant regression, $p < 0.05$, were presented)

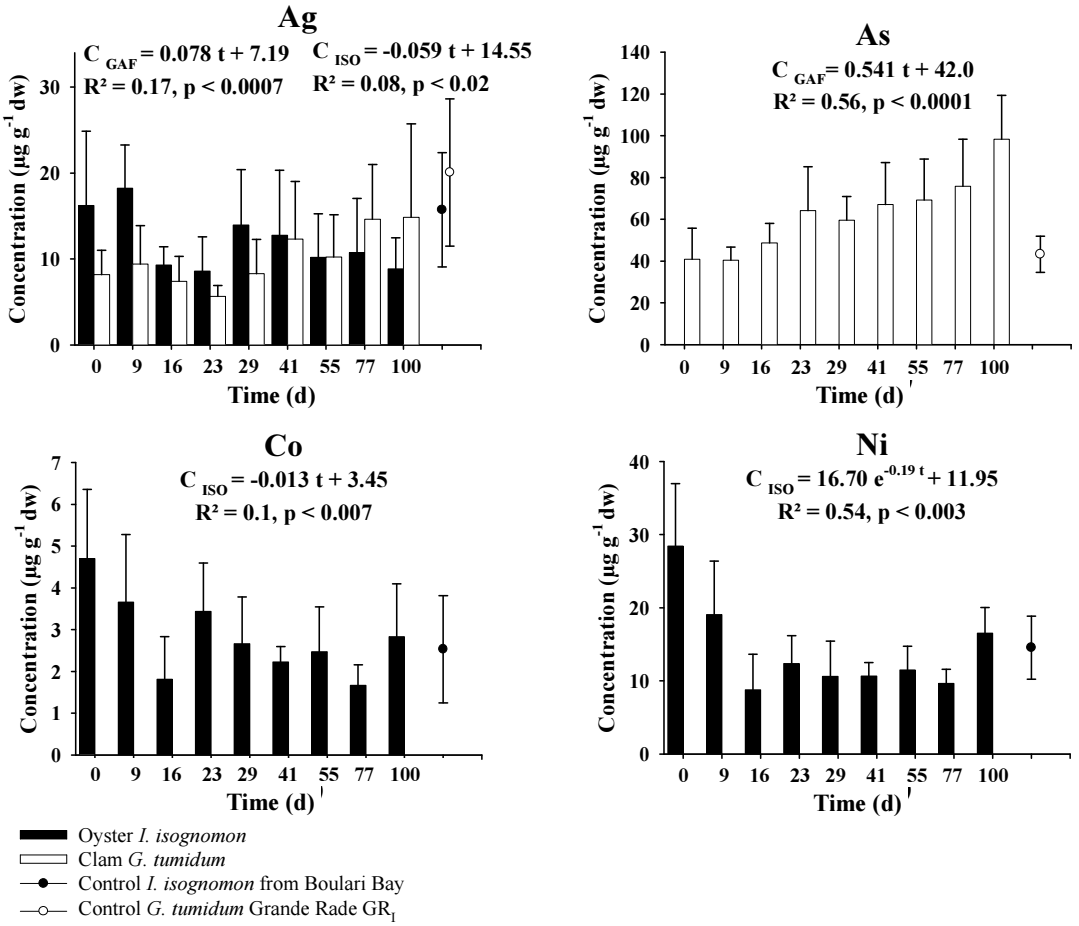


When significant increase/decrease in element concentration was observed, concentrations in transplanted organisms were compared to those of resident organisms. Statistical analyses indicated that at the end of the experiment, Ag, Cd, Cr and Cu concentrations in clams transplanted to GR_I were significantly lower (p_{Tukey} always ≤ 0.005, except for Ag, $p = 0.047$) than in resident clams from GR_I (up to 3.9 fold lower for Cd and Cr). No significant difference was found for Co, Ni and Zn concentrations between transplanted and resident clams.

At the end of the experiment, Ag concentrations in clams transplanted to Ouano Beach were significantly higher ($p_{Tukey} = 0.0001$) than those in resident clams from Ouano Beach, whereas for As, the inverse result was observed ($p_{Tukey =} 0.0003$).

Figure 3. Element concentrations (mean ± SD; µg g-1 dry wt; n = 7 for transplanted organisms and n = 5 for control organisms) in clams *Gafrarium tumidum* and oysters *Isognomon isognomon* transplanted from the contaminated stations, Grande Rade and Boulari Bay, to the reference stations, Ouano Beach and Maa Bay, respectively.

(only data shown a significant regression, $p < 0.05$, were presented)



III.1.3. Oysters *I. isognomon*

At the starting date of the experiment, concentrations of all elements in oysters from Boulari Bay were significantly higher (p_{Tukey} always ≤ 0.0002, except for Zn p = 0.006) than those collected from Maa Bay, except for As, Cd and Mn, for which no significant difference was found.

Resident populations of *I. isognomon* from Maa Bay and Boulari Bay did not exhibit significant variation in concentrations of any elements during the experiment time course.

In oysters transplanted to the contaminated station, Boulari Bay, the concentrations of Cr, Cu and Ni showed significant linear increase (R^2 = 0.14-0.24) with time.

In oysters transplanted to the clean station, Maa Bay, Ag, Co and Ni concentrations in oyster showed significant linear decrease (k_e: 0.059, 0.013 and 0.19 d^{-1}, and R^2: 0.08, 0.1 and 0.54, respectively).

At the end of the experiment, Ni concentrations in oysters transplanted to Boulari Bay were significantly lower (p_{Tukey} = 0.046) than those in resident oysters from Boulari Bay. No significant difference was found for Cr and Cu.

The concentrations of Ag, Co and Ni at the end of the experiment were significantly higher (p_{Tukey} always ≤ 0.0001) in oysters transplanted to Maa Bay than those in resident oysters from Maa Bay.

III.2. TRANSPLANTATION IN GRANDE RADE

III.2.1. Sediments

Sediments collected from Ouano Beach, Maa Bay, GR1 and GR2 revealed that concentrations of all elements were significantly higher (1 to 3 orders of magnitude higher) in sediments collected from GR1 (p_{Tukey} always ≤ 0.0002) compared to the other three stations, except for As which displayed the highest concentration in GR2 (p_{Tukey} = 0.0002) (Table 2).

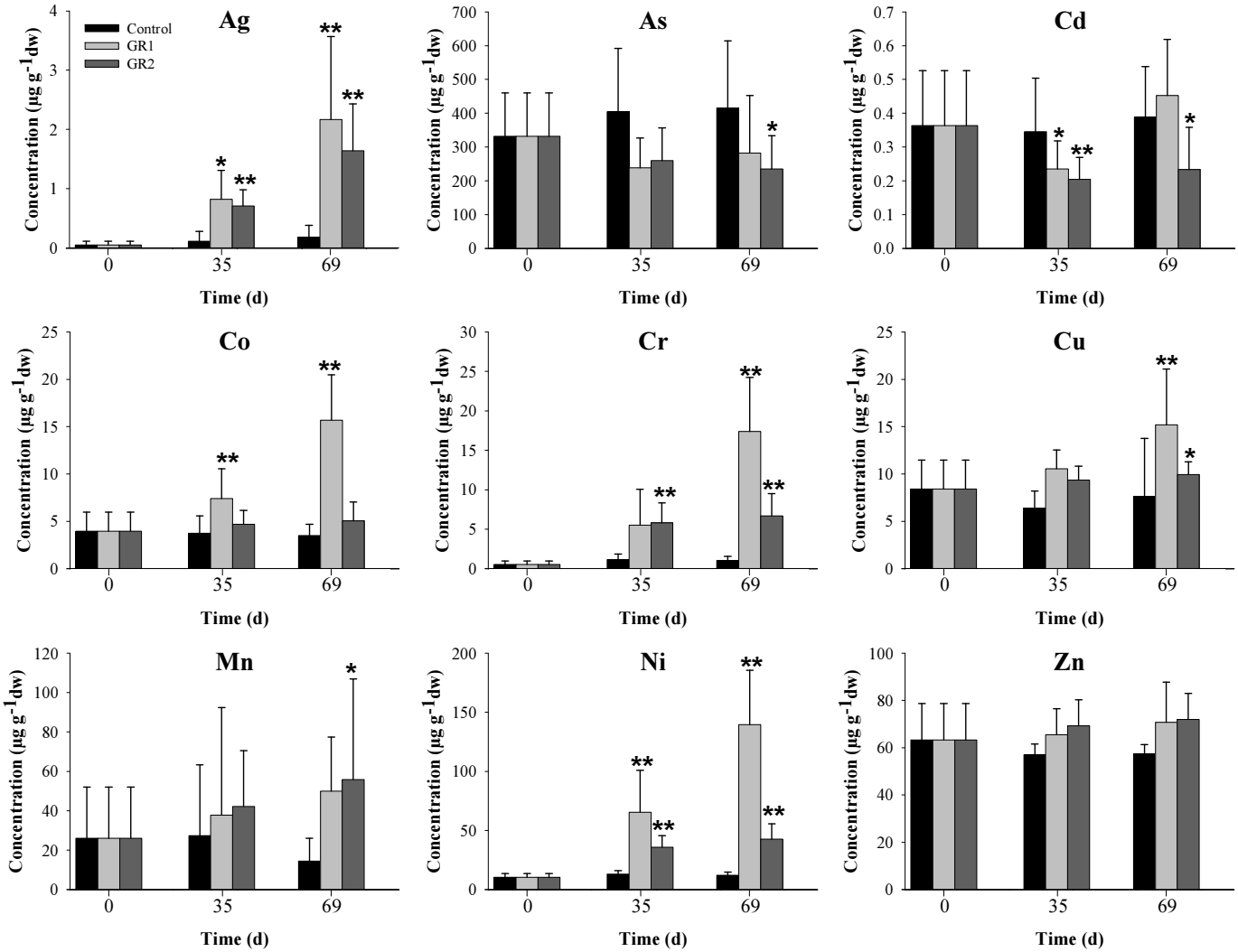
III.2.2. G. tumidum

Element concentrations in resident clams from Ouano Beach showed no significant difference with time.

At the most contaminated station GR1, Ag, Co and Ni concentrations at 35 d and 69 d were significantly higher than those measured at t_0 (p_{Tukey} always ≤ 0.0001 for Ni and ≤ 0.02 for Ag and Co) (Fig. 4) and concentrations at 69 d were significantly higher than those after 35 d of transplantation. Concentrations of Cr and Cu at 69 d were significantly higher than those at 0 and 35 d (p_{Tukey} always ≤ 0.0003), whereas no significant difference was found between the concentrations at time 0 and 35 d of transplantation. No significant difference was found between the concentration of As, Mn and Zn at 35 and 69 d.

Figure 4. Element concentrations (mean ± SD; µg g-1 dry wt; n = 30 for transplanted organisms and n = 20 for control organisms) in clams *Gafrarium tumidum* transplanted to the stations GR1 and GR2 in Grande Rade.

(* indicated that the concentration is significantly different from those at the day 0 of the concentration in organisms at 0 d; * $p < 0.05$, ** $p < 0.001$)



At the second station GR2, which displays a lower contamination than GR1 according to the element concentrations in sediment, Ag and Ni concentrations at 35 d and 69 d were significantly higher (p_{Tukey} always ≤ 0.0005) than those at t_0 and concentrations at 69 d were significantly higher than those after 35 d (p_{Tukey} = 0.0005 and 0.03 respectively). Cr concentrations at 35 and 69 d were significantly higher (p_{Tukey} always ≤ 0.0001) than those at the start of the transplantation, but no significant differences were observed between 35 and 69 d. Cu and Mn concentrations at 69 d of transplantation were significantly higher (p_{Tukey} =

0.041) than those at the starting date of the experiment. No significant difference was found for Co and Zn concentrations at 0, 35 and 69 d. In contrast, As concentration at 69 d was significantly lower than those at 0 d ($p_{Tukey} = 0.014$).

Element concentrations after 35 and 69 d of transplantation were compared between the stations GR1 and GR2. Results indicated that after 35 d, the Co, Cu and Ni concentrations in clams at GR1 station were significantly higher than those at GR2 station (p_{Tukey} always $\leq$ 0.0002, except for Cu, p = 0.01). For other elements, no significant difference between GR1 and GR2 was found after 35 d. After 69 d of transplantation, the concentrations of Cd, Co, Cr, Cu and Ni in clams at GR1 were significantly higher (p_{Tukey} always $\leq$ 0.0002) than those at GR2.

III.2.3. Oysters *I. isognomon*

Element concentrations in resident oysters from Maa Bay showed no significant variation over the duration of experiment

At the transplantion station GR1, Ni concentrations at 35 d and 69 d were significantly higher than those at t_0 and Ni concentrations at 69 d were significantly higher than those after 35 d (p_{Tukey} always $\leq$ 0.005). Concentrations of Co, Cr and Cu at 35 and 69 d were significantly higher than those at t_0 (p_{Tukey} always $\leq$ 0.0006); however no significant differences were found among these concentrations measured at 35 and 69 d. Ag concentration at 69 d was significantly higher than those at t_0 and 35 d ($p_{Tukey} = 0.03$); but, no significant difference was found between concentrations at t_0 and 35 d of transplantation. Concentrations of As, Cd, Mn and Zn exhibited no significant difference in oysters in station GR1 over the whole transplantation duration.

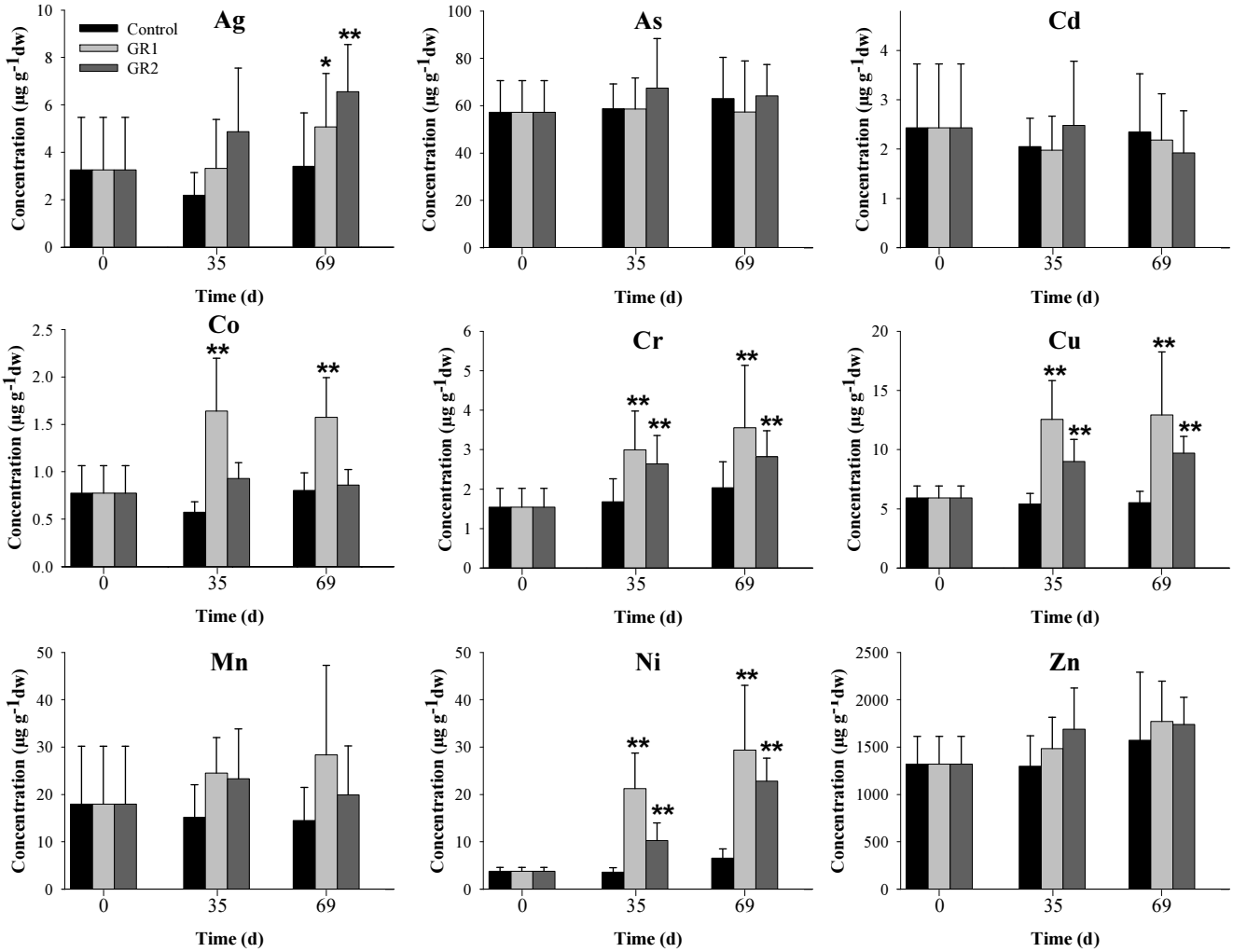
At the transplantation station GR2, Ni concentrations at 35 d and 69 d were significantly higher than those at t_0 and concentrations at 69 d were significantly higher than those after 35 d (p_{Tukey} always $\leq$ 0.0001). No significant difference was found among the concentrations of As, Cd, Co, Mn and Zn in oysters at days 0, 35 and 69. Concentrations of Cr and Cu at 35 and 69 d were significantly higher than those at t_0 (p_{Tukey} always $\leq$ 0.0001), but no significant differences were found between their concentrations at 35 and 69 d. Ag concentrations at 69 d were significantly higher than those at t_0 ($p_{Tukey} = 0.0002$) and 35 d ($p_{Tukey} = 0.02$), but no significant difference was found between concentrations at t_0 and 35 d.

After 35 d of transplantation, oysters transplanted in GR1 displayed concentrations of Co, Cu and Ni significantly higher than those at GR2 (p_{Tukey} always $\leq$ 0.0001) whereas concentrations

of Ag and Zn in GR1 oysters were significantly lower than those at GR2 (p_{Tukey} = 0.02 and 0.048, respectively). After 69 d of transplantation, concentrations of Co, Cr, Cu, Mn and Ni were significantly higher in oysters transplanted at station GR1 than those at GR2 (p_{Tukey} always ≤ 0.002 for Co and Cu, and $p < 0.04$ for Cr, Mn and Ni). Ag concentrations at GR1 were significantly lower than those at GR2 (p_{Tukey} = 0.009).

Figure 5. Element concentrations (mean ± SD; µg g-1 dry wt; n = 30 for transplanted organisms and n = 20 for control organisms) in oysters *Isognomon isognomon* transplanted to the stations GR1 and GR2 in Grande Rade.

(* indicated that the concentration is significantly different from those at the day 0 of the concentration in organisms at 0 d; * $p < 0.05$, ** $p < 0.001$)



III.3. ESTIMATION OF THE MINIMUM DETECTABLE DIFFERENCE OF CONCENTRATIONS

A power analysis was performed to determine the minimum sample size necessary to detect significant difference ($p < 0.05$) between concentrations of a given element in two groups of organisms. The variability of the data depends upon the element, the species, the stations and the concentration levels. The highest variance was observed in the samples displaying the highest concentration, consequently, minimum and maximal variance of the transplanted batches were used to determine the range of minimal sample size necessary to detect given differences of concentrations with statistical significance. Considered differences of concentrations were selected to be representative of those that are actually encountered in the field (Table 3). Results indicate that the sample size depends on the element investigated. Generally, a sample of size ≥ 50 organisms would be required to detect realistic differences in element concentrations, ranging from 0.5 (Cd) to 150 (As) $\mu g\ g^{-1}$ dry weight.

Table 3. Minimal sample size of the clam *G. tumidum* and the oyster *I. isognomon* necessary to detect with 90 % significant difference ($p < 0.05$) of concentrations between two groups of organisms.

Observed range of element concentrations represents concentrations that have been measured in the two species resident from different stations along the New Caledonia coast, number between brackets represents concentrations that have been reached during transplantation experiments.

Metal	Species	Observed range Concentration*	Difference ($\mu g\ g^{-1}$ dry wt)	Sample size *G. tumidum* Concentration		Sample size *I. isognomon* Concentration	
				Low	**High**	**Low**	**High**
Ag	Clam	0.02-33.1	**1**	< 3	43	21	110
	Oyster	1.5 – 32.8	**3**	**< 3**	**6**	**4**	**14**
			10	**< 3**	**< 3**	**< 3**	**< 3**
			30	**< 3**	**< 3**	**< 3**	**< 3**
As	Clam	37.4-441	**10**	3713	8260	32	111
	Oyster	21.6-76.6	**20**	921	2065	**9**	**29**
			40	231	517	**4**	**8**
			80	59	130	**< 3**	**< 3**
			150	**18**	**38**	**< 3**	**< 3**
			350	**5**	**8**	**< 3**	**< 3**
Cd	Clam	0.17-1.8	**0.2**	4	160	220	894
	Oyster	1.2-2.5	**0.5**	**< 3**	**27**	36	144
			1	**< 3**	**8**	**10**	**37**
			2	**< 3**	**4**	**4**	**10**
Co	Clam	1.1-7.2 (15.7)	**0.2**	780	> 10000	8	170
	Oyster	0.5-2.5	**0.5**	126	1945	**< 3**	**28**
			1	32	487	**< 3**	**8**
			2	9	122	**< 3**	**< 3**
			5	**< 3**	**21**	**< 3**	**< 3**
			10	**< 3**	**6**	**< 3**	**< 3**
Cr	Clam	1.1-10.5 (17.4)	**1**	7	993	**9**	**54**
	Oyster	1.6-9	**2**	< 3	248	**4**	**15**
			4	**< 3**	**63**	**< 3**	**5**
			8	**< 3**	**17**	**< 3**	**< 3**
			15	**< 3**	**8**	**< 3**	**< 3**
Cu	Clam	5.6-88.2	**2**	15	184	6	153
	Oyster	3.1-17.3	**4**	**5**	**47**	**< 3**	**39**
			8	**< 3**	**12**	**< 3**	**11**
			15	**< 3**	**5**	**< 3**	**4**
			30	**< 3**	**< 3**	**< 3**	**< 3**
			60	**< 3**	**< 3**	**< 3**	**< 3**
Mn	Clam	5.5-187.4	**2**	727	8590	260	1938
	Oyster	17.0-34.7	**4**	183	2148	66	485
			8	47	538	18	122
			15	14	154	**6**	**36**

			30	**5**	**40**	**< 3**	**10**
			60	**< 3**	**11**	**< 3**	**4**
			120	**< 3**	**4**	**< 3**	**< 3**
Ni	Clam	8.1-63.2 (140)	**2**	36	6505	7	963
	Oyster	2.2-16.0 (32.4)	**4**	10	1627	< 3	242
			8	4	410	**< 3**	**62**
			15	< 3	117	**< 3**	**19**
			30	**< 3**	**30**	**< 3**	**5**
			60	**< 3**	**9**	**< 3**	**< 3**
			120	**< 3**	**4**	**< 3**	**< 3**
Zn	Clam	55.6-154	**5**	14	248	>10000	>10000
	Oyster	1718-13817	**10**	**5**	**63**	722	4180
			50	**< 3**	**4**	182	1045
			100	**< 3**	**< 3**	**9**	**43**
			500	**< 3**	**< 3**	**4**	**12**
			10000	**< 3**	**< 3**	**< 3**	**< 3**

* Breau 2003; Hédouin et al. submitted-a, Present study

IV. DISCUSSION

This field study has investigated the field accumulation and depuration of 9 selected elements in two tropical bivalves in order to validate their usefulness as bioindicator species. Element concentrations in resident control organisms from each site showed no significant variation with time during the transplantation time course, indicating that any increases (or decreases) of element levels in tissues of transplanted individuals would actually reflect a higher (or a lower) metal contamination level in the studied station, and should not be due to influence of seasonal and/or physiological factors.

When the clams and oysters were transplanted in their respective contaminated site, the uptake of the selected elements displayed different trends. Indeed, at the end of the transplantation period, observed concentrations in organisms were either lower or similar than those measured in resident populations of the contaminated site, or did not change compared to their initial concentration levels.

Concentrations of Co, Ni and Zn in clams and Cr and Cu in oysters reached values similar to those measured in resident organisms. Similar findings have been previously reported for Zn and Cu in the soft tissues of the mussel *M. edulis* transplanted to a temperate polluted Bay (Roesijadi et al. 1984). However, since observed uptake displayed linear kinetics over the transplantation duration, these concentrations could most probably have continued to increase if the observation duration was longer. This is supported by the observations made in the

second transplantation experiment, in which clams transplanted to GR1 and GR2 displayed Co and Ni concentrations (up to 15.7 ± 4.8 and 139.5 ± 45.8 μg g^{-1} dry wt, respectively) exceeding those of the resident clams from Grande Rade (7.2 ± 2.3 and 63.2 ± 13.5 μg g^{-1} dry wt for Co and Ni, respectively).

In contrast, concentrations of Ag, Cd, Cr, Cu in transplanted clams and Ni in transplanted oysters significantly increased during the transplantation period but did not reach the values measured in resident organisms. Taking into account the estimated uptake rate constants of these elements in clams and oysters, it can be estimated that reaching the resident concentrations would require for example approximately 35 months for Cd in clams and about 6 months for Ni in oysters. Comparable results have been previously reported in the oysters *Crassostra rhizophorae* (Wallner-Kersanach et al. 2000), the clam *Macoma balthica* (Cain & Luoma 1985) or the mussel *M. edulis* from Greenland (Riget et al. 1997). However, our results from the second transplantation experiment indicated that when both species were transplanted to a more contaminated site (GR1), accumulation of Cr in clams and Ni in oysters was faster than during the first transplantation experiment. Therefore, the slow uptake rate of Cr in clams and of Ni in oysters observed in the latter transplantation would rather be related to low bioavailability of these two metals in the contaminated site than to low bioaccumulation efficiency of the organisms.

In the case of As and Mn in clams, and Ag, As, Cd, Co, Mn and Zn in oysters, concentrations did not show significant increase during the transplantation. Similar observation were made for Cd and Zn concentrations in *Crenomytilus grayanus* after two months of transplantation (Shulkin et al. 2003). Our results suggest either that these elements were not bioavailable for the bivalves, or that clams and oysters have efficient regulation mechanisms preventing these metals to be accumulated. However, when organisms were transplanted to GR1 and GR2, concentrations of Mn in clams and of Ag and Co in oysters were actually bioaccumulated, supporting thus the low bioavailability hypothesis, at least for Mn in Grande Rade GR_I and Ag and Co in Boulari Bay.

When organisms were transplanted to clean station, the concentrations of all elements in both bivalves were almost the same after 100 d of transplantation, except for Ag, Co and Ni in oysters, which showed a low but significant decrease with time. However, Ag, Co and Ni concentrations in oysters did not reach the low concentration found in natural resident populations at the end of experiment. Such incomplete metal elimination has been reported by several authors when organisms from polluted area were transplanted to clean area (e.g. Zn in

mussels *Mytilus edulis,* Simpson 1979; Roesijadi et al. 1984; Cd and Cu in oyster *Crassostrea gigas*, Geffard et al. 2002 ; Zn, Cr and Cu in clam *Mercenaria mercenaria*, Behrens & Duedall 1981). The biological half life ($T_{b½}$) of elements investigated has been previously estimated from radiotracer experiments in *G. tumidum* and *I. isognomon* (Hédouin et al. submitted-d; Hédouin et al. to be submitted-a, to be submitted-b). Although, elements like Ag, Cd, Ni and Zn were very efficiently retained with $T_{b½} \geq 5$ months, the other elements displayed $T_{b½}$ ranging from 1 to 3 months in both bivalve species, whatever the uptake pathway considered (seawater, food or sediment). Comparison of the data indicates that in the field, depuration processes would take longer for some metals than those previously estimated from laboratory experiments. This suggests that laboratory results cannot always be extrapolated directly to environmental situations probably due to the complexity of the processes involved in metal elimination. Since the clams and the oyster showed low depuration for most of the contaminants studied, bivalve tissues would be able to retain information of contamination events over long periods of time. Nevertheless, this suggests that (1) the element concentrations in transplanted organisms are not actually able to reflect the lower contamination levels occurring in the considered station over a medium-scale period of time (3 months), and (2) the element concentrations in organisms collected from natural areas can reflect past contamination no longer occurring. Depuration may be influenced by the previous history of contaminated organisms. Wallner-Kersanach et al. (2000) showed that Cu was more easily eliminated (30 % after 30 d) in oysters that had been transplanted temporarily to a metal-rich area Cotegipe Channel for 60 d, than in oysters previously living permanently in the same contaminated area (decrease limited to 9 % after 30 d) after being returned to clean area. This suggests that specimens from the more polluted area, which are exposed to high metal concentrations, may have produced e.g. more metal binding proteins such as metallothioneins, which are involved in the sequestration and detoxification of some metals for longer periods of time (e.g. Bebianno & Langston 1992; Mason & Jenkins 1995). Such adaptation mechanisms could occur in both selected species, and hence explain the efficient high retention observed in the field. Therefore, further studies must be addressed to study the long-term elimination of elements in both bivalves from contaminated sites and from clean sites, which would be previously exposed to contaminants in the field for 2-3 months, before being transplanted in clean sites. Such experiments could thus indicate whether the previous history of contamination of the organisms plays a role in the efficient retention of elements observed in the field.

In the second transplantation experiment, element concentrations in sediment clearly indicated that the GR1 is the most contaminated station, reaching very high level of Co, Cr, Mn, Ni (up to 10,450 µg Ni g^{-1} dry weight). Ag, Co, Cr, Cu and Ni were efficiently accumulated in both transplanted clams and oysters. In addition, our results indicated that bioaccumulation is dependent on the time of exposure, sampling station and species. Indeed, the discrimination of the contamination levels between two stations was easier when organisms were transplanted for a longer time (69 d). For example, our results showed that whereas the concentrations of 5 elements in bivalve tissues (Cd, Co, Cr, Cu and Ni in clams, Co, Cr, Cu, Mn and Ni in oysters) were significantly higher in GR1 than in GR2 at 69 d; at 35 d, this difference was observed for only 3 elements (Co, Cu and Ni).

In this second transplantation experiment, clams and oysters were transplanted into the same stations and hence underwent the exposure under the same conditions. Their bioaccumulation capacities can thus be directly compared. Clams were more efficient in bioaccumulating the selected elements than oysters (e.g. concentrations measured after 69 d of transplantation increased up to a factor 7 in oysters and up to a factor 40 in clams compared to those measured at 0 d). These findings could be surprising considering previous results from laboratory radiotracer studies (Hédouin et al. to be submitted-a, to be submitted-b), which indicated a much more efficient bioconcentration in oysters than in clams when exposed to dissolved elements (CF several orders of magnitude higher). Such a difference between seawater laboratory exposures and field global exposure studies strongly suggests that the seawater pathway would not be the one driving global contamination of these organisms. Rather, ingestion of suspended particles would be the main metal uptake pathway. This could be indeed explain the higher metal levels in *G. tumidum* which lives in the sediments and feeds mainly on organic- (and metal) rich particles at the seawater-sediment interface.

In order to obtain accurate and reliable data in biomonitoring programmes, the determination of optimal sample size to be collected is fundamental information. In this context, the present study has investigated the minimum sample size necessary to detect given difference of concentrations (from 0.5 µg g^{-1} dry weight). Results are shown in Table 3 and indicated that the detection of the smallest difference between mean concentrations requires the largest sample size. Relatively large variability of concentrations in organisms within a site has frequently been reported (e.g., Gordon et al. 1980; Daskalakis 1996). In the present study, the concentration variability increased when mean concentration increased. Consequently, the sample size would increase to detect small concentration differences among organisms with

higher metal levels. Nevertheless, it is important to keep in mind that to be feasible, the sample size required in a biomonitoring programme should remain realistic.

Compared to the actual levels occurring in New Caledonia from low to high degree of contamination, the minimum difference in concentrations detectable with sample sizes of 50-60 organisms would allow an efficient discrimination of sampling stations sustaining bivalve populations. For example, a difference of 2 μg Ni g^{-1} dry weight can be detected with a sample size of 36 clams and 7 oysters in population showing low Ni levels. However, 30 clams and 62 oysters would be necessary to detect difference of 30 and 8 μg g^{-1} dry weight, respectively in population characterized by high Ni concentrations (Table 3). A sample size of 50-60 organisms has been already recommended by others authors in order to facilitate the detection of significant change in concentrations (Gordon et al. 1980; Topping 1983). In current biomonitoring programmes, organisms collected (20 oysters and 30 mussels for the NOAA Mussel Watch, Beliaeff et al. 1998; 10 oysters and 50 mussels for the French RNO, Claisse 1989) are pooled before analysis in order to reduce costs of sample preparation and analysis. However, pooling implies the loss of statistical information mainly related to the inter-individual variability, which is a main issue to assess significance of concentration differences among samples. These economic constraints are obvious in the case of large national and international biomonitoring programmes, which take into account numerous trace elements and organic contaminants. However, in New Caledonia which is mainly impacted by mining activities, metal and metalloids are the contaminants of major interest. Therefore, analysis costs would be reducing compared to biomonitoring also considering very expensive –organic contaminants. Hence, it would be most recommended to consider analysis of individual samples in order to obtain information on inter-individual variability and better scientifically supported decision tools.

V. CONCLUSION

This study clearly indicates that the use of the clam *G. tumidum* can be recommended to monitor geographical and temporal trends of contaminants in subtidal and intertidal stations of the New Caledonia lagoon. Biomonitoring studies using transplanted organisms would be an efficient solution to survey environmental levels of key local contaminants in areas lacking resident bivalves. The advantages of using transplanted organisms (active biomonitoring) over sampling natural populations (passive biomonitoring) are that it allows selecting

organisms of uniform initial element concentrations and of common origins and past history, ensuring comparable biological samples. However, if further studies confirm the very long element retention in these organisms, repetitive short-term transplantations should be preferred to single long-term transplantation experiments in order to prevent bias in element concentrations due to inefficient depuration and thus to long time-integration.

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DISCUSSION GENERALE & CONCLUSIONS

Le développement croissant des activités minières au Sud et au Nord de la Nouvelle Calédonie et leurs conséquences en terme de contamination métallique dans l'environnement marin constituent l'un des problèmes les plus préoccupants aujourd'hui. Il est apparu urgent de disposer d'un moyen efficace d'évaluer et de surveiller le degré de contamination présent dans l'environnement marin. Afin de fournir les éléments scientifiques nécessaires à la mise en place d'un réseau de surveillance utilisant des espèces bioindicatrices, différentes investigations ont été entreprises dans le cadre de cette thèse.

L'objectif principal de ce travail était de caractériser la valeur bioindicative d'une algue brune, *Lobophora variegata,* et de trois bivalves, le clam *Gafrarium tumidum*, et les huîtres, *Isognomon isognomon* et *Malleus regula*, afin d'évaluer la fiabilité de leur utilisation à terme en tant que bioindicateurs de contamination métallique dans le cadre de la mise en place d'un programme de biosurveillance des côtes néo-calédoniennes. Les quatre espèces répondent aux premiers critères permettant leur sélection en tant qu'espèces candidates au titre de bioindicateur. En effet, elles sont suffisamment abondantes et faciles à manipuler et, surtout, elles présentent des concentrations métalliques corporelles contrastées lorsqu'elles sont prélevées dans des sites soumis à des pressions de contamination supposément contrastées. La question est donc de savoir si l'information obtenue par la mesure des contaminants dans les organismes reflète effectivement les conditions de contamination du milieu.

Cette approche constitue une étape pilote pour la validation de ces espèces en tant que bioindicateurs de la contamination par le Co, Cr, Mn et Ni qui sont des contaminants spécifiques à la Nouvelle Calédonie, mais aussi par d'autres éléments (As, Ag, Cd, Zn) car l'implantation d'un tel programme de biosurveillance est susceptible d'être étendue à d'autres régions tropicales.

La démarche scientifique a consisté en (1) la caractérisation en laboratoire de la bioaccumulation et de l'élimination des principaux contaminants métalliques dans l'algue brune *L. variegata*, le clam *G. tumidum,* et les huîtres *I. isognomon* et *M. regula,* et la détermination des paramètres pouvant influencer la bioaccumulation des métaux, (2) la validation du choix des organismes *in situ*, et (3) la proposition d'une stratégie de surveillance à mettre en place dans l'avenir. Lors de la validation de terrain, deux aspects de la biosurveillance ont été testés : la biosurveillance passive, où les espèces sont collectées dans différents sites présentant des états de contamination variés et la biosurveillance active, où les espèces provenant de zones faiblement contaminées sont transplantées dans des sites où elles ne sont pas forcément présentes naturellement.

I. CARACTERISATION EN LABORATOIRE DE LA VALEUR BIOINDICATIVE DE *L. VARIEGATA*, *G. TUMIDUM*, *I. ISOGNOMON* ET *M. REGULA*

Cette étude a permis de caractériser le comportement bioaccumulateur des espèces sélectionnées en conditions contrôlées de laboratoire pour l'Ag, As, Cd, Co, Cr, Mn, Ni et Zn (***Chapitre 1***). Au cours de ces expérimentations, une attention particulière a été portée à l'influence de la voie dissoute sur la bioconcentration des contaminants dans les organismes (***Chapitres 4, 5 et 6***) en raison de l'utilisation d'un nouveau procédé appelé HPAL pour « High Pressure Acid Leach » par l'industrie Goro-Nickel. Le suivi de l'usine pilote de Goro-Nickel a en effet montré que l'effluent issu de ce procédé contenait les métaux principalement sous formes dissoutes (e.g. Cr, Mn, Ni), contaminants qui seront par la suite rejetés dans le canal de la Havannah (Rescan 2001; Goro-Nickel 2003; Stauber et al. 2003).

Pour être informative, une espèce bioindicatrice doit renseigner sur l'état de contamination du milieu dans lequel elle vit (Phillips 1990), et doit par conséquent bioaccumuler les métaux de façon proportionnelle à leur concentration présente dans le milieu ambiant. Bien qu'il s'agisse probablement là ***du*** critère que doit nécessairement rencontrer un organisme pour pouvoir être considéré et utilisé comme bioindicateur, extrêmement peu d'études se sont « attardées » à vérifier cette propriété (e.g. Miramand et al. 1980; Bjerregaard 1988; Warnau et al. 1997). Il semble en effet que la tendance est plutôt de considérer que les espèces utilisées comme indicateurs se conforment *de facto* à ce pré-requis, ce qui relève très généralement de l'usage d'une solution de facilité plutôt que d'un comportement scientifique (e.g., Phillips 1990; Warnau 1996).

Nos expériences en laboratoire ont permis de montrer que pour des gammes de concentrations réalistes sur le plan environnemental (i.e. correspondant aux concentrations effectivement rencontrées dans le milieu), les cinétiques d'accumulation (facteurs de concentration, FC, en fonction du temps) et d'élimination (% résiduel de la concentration initiale) du Ni chez l'algue *L. variegata,* le clam *G. tumidum*, et les deux huîtres *I. isognomon* et *M. regula* sont peu influencées par la concentration en Ni dissout sur toute la gamme de concentration testée (jusqu'à 1400 ng de Ni ajouté l^{-1}), bien que l'huître *I. isognomon* présente des capacités de bioconcentration plus faibles à la plus forte concentration de Ni testée (***Chapitres 4 et 6***). Pour

les autres éléments étudiés (As, Cd, Co, Cr, Mn, Zn), le comportement bioconcentrateur ainsi que la distribution tissulaire et sub-cellulaire des éléments dans les organismes sont identiques pour les gammes de concentration étudiées à l'exception du Co et du Zn dans *G. tumidum* et *I. isognomon*, pour lesquels une diminution du FC aux fortes concentrations est observée (***Chapitres 5 et 6***). D'un point de vue pratique, l'existence d'une concentration seuil pour le Co et le Zn, à partir de laquelle la proportionnalité entre la concentration métallique dans les tissus et dans l'eau de mer n'est plus respectée ne remet pas en cause l'utilisation de ces organismes comme bioindicateurs du Co et du Zn dans les eaux calédoniennes, car la proportionnalité est préservée sur la gamme de concentrations maximales de Co et Zn effectivement mesurées dans les sites les plus contaminés du lagon de Nouvelle Calédonie (entre 14 et 20 ng Co l^{-1} et 1500 ng Zn l^{-1}, Goro-Nickel 2004). Même si les concentrations métalliques rencontrées en Nouvelle Calédonie sont extrêmement élevées, il n'en demeure toutefois pas moins vrai que, au cas où l'utilisation de ces organismes serait étendue à d'autres zones tropicales, une étude préalable serait nécessaire pour s'assurer que les concentrations maximales présentes dans le milieu ne soient pas supérieures à celles considérées ici et ce, afin de s'assurer de la fiabilité de l'information qui serait fournie par les indicateurs dans le cadre du Co et du Zn.

Les capacités de bioconcentration des organismes sélectionnés varient en fonction de l'élément et de l'espèce considérée, dans l'ordre décroissant : algue > huîtres > clam (tous éléments confondus). Bien que les huîtres *I. isognomon* et *M. regula* présentent une capacité de bioconcentration plus importante que celle du clam *G. tumidum*, en terme de rapidité de détection et de capacité de bioconcentration d'un événement contaminant, parmi les quatre organismes étudiés c'est l'algue brune *L. variegata* qui apparaît comme l'espèce la plus indiquée en tant que bioindicateur de la contamination dissoute.

Afin de prendre en compte de manière plus réaliste les processus conduisant à la bioaccumulation des métaux dans les organismes sélectionnés, la voie trophique et la voie sédimentaire ont également été considérées dans les expériences en laboratoire (***Chapitres 2, 3, 4 et 7***). Le phytoplancton, à la base de la chaîne alimentaire, est capable de concentrer certains métaux à partir de la phase dissoute jusqu'à plus de 10^5 fois la concentration du milieu (e.g. Fisher & Reinfelder 1995). Composant de la nourriture des bivalves, le phytoplancton peut constituer dès lors une source de contamination métallique significative pour les échelons trophiques supérieurs (e.g. Fisher & Reinfelder 1995). Du point de vue de l'influence de la concentration métallique associée au phytoplancton, les résultats obtenus

montrent que les bivalves *G. tumidum* et *I. isognomon* assimilent et retiennent le Co associé au phytoplancton de manière similaire quelque soit la concentration métallique (jusqu'à 500 ng Co ajouté l^{-1}) à laquelle le phytoplancton a été préalablement exposé (***Chapitre 7***). Bien que des résultats similaires aient été observés concernant l'assimilation du Se ingéré via des diatomées (*Thalassiosira pseudonana*) dans la moule *Mytilus edulis* (Wang & Fisher 1996b), cette tendance ne reflète pas un comportement général. Wang & Fisher (1996) considèrent en effet que la réponse de la moule *M. edulis* aux variations de concentrations métalliques dans la nourriture ingérée est dépendante de l'élément étudié. L'efficacité d'assimilation -EA- du Zn dans la moule diminue aux concentrations métalliques élevées alors qu'une relation inverse est observée pour le Cd. Bien que, dans notre étude, l'influence de la concentration métallique associée au phytoplancton n'a pas pu être testée pour d'autres éléments, les résultats obtenus via la voie alimentaire pour *G. tumidum* et *I. isognomon* corroborent ceux préalablement obtenus via la voie dissoute (***Chapitre 5***). Les deux bivalves étant capables d'accumuler les contaminants présents dans l'eau de mer et associés aux cellules phytoplanctoniques sans que la concentration métallique présente ne perturbe leur comportement bioaccumulateur (via e.g. la mise en place d'un mécanisme de régulation), l'information relative à un évènement contaminant fournie par *G. tumidum* et *I. isognomon* sera proportionnelle à son intensité.

Contrairement aux données concernant la concentration métallique associée au phytoplancton, il a été établi que la qualité et la quantité de nourriture, à laquelle les organismes étaient exposés, influençaient de manière notable l'EA des métaux (***Chapitre 7***). Les expériences en laboratoire ont en effet montré que l'EA des métaux associés aux cellules phytoplanctoniques diminuait avec des concentrations algales croissantes, et les métaux associés à l'espèce phytoplanctonique *Isochrysis galbana* étaient de manière générale les mieux assimilés. Cependant, l'étude de la bioaccumulation des métaux par la voie trophique en laboratoire est basée sur l'utilisation de cultures mono-spécifiques où chaque condition alimentaire est testée séparément (e.g. concentration cellulaire, espèce phytoplanctonique). Il est évident que ces conditions expérimentales de laboratoire sont éloignées de celles des milieux aquatiques naturels. Spécifiquement, dans le lagon Sud-Ouest de Nouvelle-Calédonie, la structure et la composition du phytoplancton varient sur une échelle spatiale et temporelle (Jacquet et al. in press) et sont étroitement liées au rôle structurant joué par les apports en azote (Jacquet 2005). Cependant, ces apports ne constituent qu'un des effets des activités anthropiques et Jacquet et al. (in press) ont observé que la composition phytoplanctonique le long de deux radiales côte-

large sous influence anthropique urbaine (baie de Sainte-Marie) et industriel (Grande Rade) était totalement différente alors que la charge en éléments nutritifs était comparable. Les apports métalliques pourraient donc jouer un rôle sur la structure et la composition des communautés phytoplanctoniques. Bien que l'étude de la bioaccumulation des métaux par la voie trophique en laboratoire ne permette pas de refléter parfaitement les conditions du milieu naturel, ces données apportent les premières valeurs sur l'EA des principaux contaminants du lagon de Nouvelle-Calédonie (en particulier pour le Ni qui est relativement peu étudié en milieu marin) chez *G. tumidum* et *I. isognomon* et montrent l'existence d'une forte variabilité de cette EA des métaux en fonction des conditions alimentaires testées en laboratoire. A ce titre, ces données constituent une base de référence pour de futures études nécessaires à la compréhension de la bioaccumulation des métaux par la voie trophique *in situ*. La part associée à la voie alimentaire étant d'autant plus importante que l'EA des éléments est élevée (e.g. Wang & Fisher 1997a), une des voies de recherche qui mériterait donc d'être abordée dans la suite de ce travail serait de déterminer l'influence des changements dans les communautés phytoplanctoniques sur la contribution de la voie alimentaire dans la bioaccumulation globale des métaux chez *G. tumidum* et *I. isognomon* notamment.

Les expériences réalisées en laboratoire concernant la voie sédimentaire montrent que la bioaccumulation des métaux liés aux sédiments par les bivalves est faible avec des facteurs de transfert à l'équilibre - TF_{SS} - *in toto* généralement < 1 (***Chapitres 2 et 3***). De plus, la comparaison des FC et des FT dans les bivalves, représentatifs de la bioaccumulation des contaminants par la voie dissoute et particulaire, respectivement, montre une biodisponibilité des métaux liés aux sédiments beaucoup plus faible que celle des métaux dissouts et suggère dès lors que la voie sédimentaire serait minoritaire dans la bioaccumulation totale des contaminants dans les organismes sélectionnés. Cependant, la seule comparaison du FC et du FT est restrictive dans le sens où seule la capacité de bioaccumulation des métaux particulaires/dissouts est prise en considération et cette comparaison ne tient pas compte des concentrations des deux sources existant dans la nature (Luoma 1989). Or les résultats obtenus *in situ* montrent généralement des concentrations en contaminants dans le compartiment sédimentaire supérieures de plusieurs ordres de grandeurs par rapport à celles présentes dans l'eau de mer. Ainsi, il convient d'examiner l'importance de la bioaccumulation de chaque voie en terme de contribution relative en considérant les capacités de bioaccumulation des organismes vis-à-vis d'une voie d'accumulation ainsi que les

concentrations existant *in situ*, ou du moins les correspondances entre les concentrations des différents compartiments (e.g. K_d, constante de distribution des métaux entre le compartiment sédimentaire et dissout). De plus, l'intégration des informations cinétiques relatives aux trois voies d'exposition étudiées (eau de mer, nourriture et sédiments) s'avère difficile à gérer de façon intégrée. La procédure suivie pour déterminer les paramètres cinétiques relatifs à la bioaccumulation via l'eau et les sédiments d'une part et la nourriture d'autre part n'étant pas directement comparables, il n'est pas possible d'évaluer directement la contribution de chaque voie d'accumulation dans la bioaccumulation totale. Pour cela, l'utilisation des données relatives aux cinétiques d'accumulation et d'élimination via les 3 voies d'exposition acquises en laboratoire et leur intégration dans un modèle de bioaccumulation globale initialement proposé par Landrum et al. (1992) puis repris par Thomann et al. (1995) devrait permettre une meilleure estimation de l'importance de chaque voie d'accumulation des métaux dans les organismes. L'utilisation de ce modèle a permis de mieux appréhender et comprendre les processus de bioaccumulation par une approche globale, et le compartiment particulaire est désormais reconnu comme une voie d'accumulation importante par de nombreux auteurs (e.g. Luoma et al. 1992; Wang et al. 1996; Griscom et al. 2002b). En effet, le couplage d'expérience en laboratoire et *in situ* et la compilation des résultats dans un modèle de bioaccumulation a montré que 98 - 99 % du Se mesuré dans le clam *Macoma balthica* en baie de San Francisco pouvaient provenir de l'ingestion du Se via la voie particulaire (Luoma et al. 1992). Ainsi, la détermination des contributions relatives des différentes voies d'accumulation dans la bioaccumulation globale des contaminants dans les organismes, ainsi que la considération des facteurs biotiques/abiotiques influençant la bioaccumulation des métaux sont essentielles pour une meilleure compréhension et interprétation des résultats issus des programmes de biosurveillance.

Les deux huîtres indopacifiques *I. isognomon* et *M. regula* sélectionnées dans cette étude bien que possédant un phénotype et un mode de vie relativement similaires (Yonge 1968), présentent des comportements de bioaccumulation extrêmement semblables vis-à-vis des métaux étudiés, à l'exception de l'Ag et, dans une moindre mesure, du Cd et du Ni (***Chapitres 2 et 4***). En effet, l'accumulation et la rétention de l'^{110m}Ag par la voie dissoute et par la voie trophique via les cellules phytoplanctoniques sont beaucoup plus efficaces dans les tissus d'*I. isognomon* que chez *M. regula*. Cette accumulation particulière de l'Ag par l'huître *I. isognomon*, également observées chez certains individus de l'espèce *G. tumidum* considérés comme des organismes hyper accumulateurs (***Chapitre 3***), peut être liée à l'existence d'un

mécanisme de détoxication de ce métal qui provoquerait son immobilisation et son stockage sur le long terme. Un tel processus d'immobilisation de l'Ag sous forme stable et non toxique est bien connu chez différentes espèces de bivalves. En effet, les Pectinidae et les Ostreidae accumulent dans leur tissus mous des concentrations en Ag très élevées, dont une fraction importante est stockée sous forme d'Ag_2S amorphe (e.g. Martin & Kim 1977; Martoja et al. 1988; Martoja et al. 1989; Berthet et al. 1990; Berthet et al. 1992). Cette forme détoxifiée de l'argent est précipitée sous les membranes basales des cellules de la plupart des organes pour une très longue durée car les composés d'Ag_2S sont insolubles et forment des granules qui sont éliminés très lentement (Berthet et al. 1990; Berthet et al. 1992). Une différence entre les deux huîtres a également été mise en évidence pour le Ni. Même si elles présentent des capacités de bioconcentration similaires lorsque l'on considère la totalité des tissus mous, à l'échelle des organes, il s'avère que les branchies d'*I. isognomon* présentent une capacité d'accumulation beaucoup plus élevée que *M. regula* (***Chapitre 4***). Pour le Cd, l'huître *I. isognomon* présente des capacités de bioconcentration plus élevées que l'huître *M. regula.* Ainsi, les deux espèces, bien que très proches sur le plan morphologique et partageant la même niche écologique, présentent donc des mécanismes permettant la détoxication de certains métaux (Ag, Cd et Ni) qui peuvent être différents. Dans l'optique de la surveillance des contaminants d'origines minières, de part leur comportement de bioaccumulation relativement similaire, les deux huîtres peuvent être utilisées de manière pratiquement interchangeables, bien qu'une préférence soit accordée à *I. isognomon*, du fait de ses capacités de bioaccumulation généralement supérieures à *M. regula*, quand différence il y a. Par contre, dans le cadre d'un suivi de la contamination domestique et urbaine autour de grandes villes comme Nouméa, par l'intermédiaire du suivi des concentrations en Ag, qui est reconnu comme un indicateur de la contamination domestique (Sanudo-Willhelmy & Flegal 1992), l'utilisation simultanée des deux huîtres apparaît judicieuse. En effet, alors que les fortes capacités de bioaccumulation et de rétention de l'huître *I. isognomon* en font un bon bioindicateur à long terme de la contamination anthropique, l'huître *M. regula*, au contraire, permet d'obtenir une information sur une échelle de temps plus courte.

Les données obtenues dans le cadre de ce travail indiquent que *L. variegata, G. tumidum, I. isognomon,* et *M. regula* sont de «bons bioindicateurs» de la contamination métallique de l'environnement dans lequel ils vivent. En effet, la rapidité (k_u) et l'ampleur (FC) de l'incorporation métallique indiquent que ces organismes devraient être à même de pouvoir révéler rapidement (en quelques semaines) l'état de contamination d'un site donné. Par

ailleurs, les espèces étudiées sont de bons intégrateurs de l'information écotoxicologique dans le temps. En effet, les longues rétentions des métaux préalablement incorporés via les différentes voies d'exposition ($T_{b½}$ généralement supérieurs à 1 mois) indiquent que ces espèces sont susceptibles de préserver l'information relative à un évènement contaminant pendant un temps relativement long. Ainsi, au terme de cette première partie d'expériences réalisées en laboratoire, les espèces les plus intéressantes en tant que bioindicateurs sont certainement l'algue *L. variegata* et les huîtres *I. isognomon* et *M. regula*, de part leur forte capacité de bioaccumulation des métaux considérés, et secondairement le clam *G. tumidum.* Toutefois, le clam est une espèce consommée en Nouvelle-Calédonie par les populations locales (Baron & Clavier 1992b) et pourrait constituer une source non négligeable d'exposition aux métaux pour l'Homme. En effet, l'étude de la distribution cellulaire des métaux chez *G. tumidum* a montré que parmi les différents éléments toxiques considérés, le Cd était principalement contenu dans les fractions cytosoliques des branchies et des viscères (80 à 95 %). Or cette fraction est considérée comme hautement biodisponible pour le niveau trophique supérieur (en l'occurrence, l'Homme) (Nott & Nicolaidou 1990; Reinfelder & Fisher 1991; Ettajani et al. 2001; Wallace & Luoma 2003). L'utilisation simultanée d'espèces comestibles et non comestibles permet d'élargir le champ d'application du programme de biosurveillance des contaminants dans l'environnement marin au domaine sanitaire, en mesurant les niveaux de contaminants dans les espèces comestibles et en surveillant alors le risque pour le consommateur.

Comme le démontrent nos résultats, il est possible d'établir une correspondance entre les concentrations métalliques dans les organismes et celles dans l'eau en conditions contrôlées de laboratoire et de déterminer les conditions limitantes, c'est-à-dire les conditions au-delà desquelles les organismes peuvent modifier leur comportement bioaccumulateur vis-à-vis des éléments métalliques. Cependant, l'utilisation de telles données pour quantifier par exemple la concentration métallique dissoute dans l'environnement à partir de mesures métalliques effectuées dans les tissus des organismes reste utopique. En effet, les organismes peuvent montrer des capacités d'adaptation aux conditions environnementales (Bryan 1976; Bryan et al. 1985) ; il a par exemple été observé que le facteur de bioconcentration (BCF) des métaux dépendait de leurs concentrations dans le milieu (McGeer et al. 2003). Aux faibles concentrations, les organismes tendent à concentrer les métaux, en particulier ceux qui leurs sont essentiels, le BCF observé est alors élevé; alors qu'une augmentation de l'exposition aux métaux peut induire par exemple des mécanismes de régulation physiologique, le BCF

observé sera d'autant plus faible. Ainsi, même si les conditions de laboratoire essaient de reproduire les différentes voies d'accumulation existant dans l'environnement, ces résultats sont réduits à des conditions particulières. Pour pallier à ce problème, l'étude *in situ* de la bioaccumulation des contaminants dans les organismes permet de valider la sélection des organismes étudiés en tant que bioindicateurs fiables de la contamination métallique naturelle des eaux néo-calédoniennes en conditions contaminantes complexes, en tenant compte des variations spatiales et temporelles des facteurs environnementaux et de l'influence des conditions contaminantes sur la biologie des espèces (passé écotoxicologique).

II. VALIDATION *IN SITU*

Afin de tester la fiabilité de trois des quatre espèces sélectionnées, *L. variegata G. tumidum* et *I. isognomon* en tant d'espèces bioindicatrices, les concentrations de 9 éléments (Ag, As, Cd, Co, Cr, Cu, Mn, Ni et Zn) ont été mesurées dans des individus de ces espèces prélevées en plusieurs stations du lagon néo-calédonien (***Chapitre 8***).

L'analyse des concentrations de ces contaminants dans les trois espèces et dans les sédiments a permis de classer ces stations entre celles présentant un faible degré de contamination, appelées « stations de références », et celles présentant au contraire un fort degré de contamination. Par rapport aux données acquises concernant les concentrations en éléments dans l'algue *L. variegata* et l'huître *I. isognomon* la baie Maa peut-être considérée comme une station de « référence » et la baie de Boulari comme une station fortement contaminée pour ces 2 espèces par rapport aux autres stations, à l'exception de l'As et du Zn pour *I. isognomon* (***Chapitre 8*****).** Bien que les réponses des deux organismes, l'algue *L. variegata* et l'huître *I. isognomon* appartenant à des niches écologiques différentes mais vivant dans le même biotope, sont différentes en terme de représentativité de la fraction métallique biodisponible (algue : fraction dissoute, huître : fraction dissoute et particulaire), les réponses sont qualitativement semblables et convergent vers les mêmes conclusions. De part les faibles concentrations mesurées dans le clam *G. tumidum* et les sédiments collectés à la plage d'Ouano, la plage d'Ouano est considérée comme un site de « référence » pour tous les éléments, à l'exception de l'As alors que la Grande Rade (GR_I) est identifiée comme la station la plus contaminée pour cette espèce, avec des concentrations nettement supérieures à celle de la plage d'Ouano.

Les concentrations des éléments dans les différents organismes sont fortement influencées par leurs origines et la station d'échantillonnage a été identifiée comme le facteur expliquant le maximum de variabilité pour la plupart des éléments (e.g. Cu, Mn et Zn dans l'huître *I. isognomon*, et Ag, As, Cd, Co, Cr, Cu et Ni dans le clam *G. tumidum*). L'effet de station étant une des conditions d'utilisation des bioindicateurs, les résultats obtenus montrent que la mesure des concentrations corporelles en contaminants dans les populations résidentes de clam *G. tumidum*, d'algue *L. variegata* et d'huître *I. isognomon* permet donc de caractériser de manière efficace le degré de contamination des stations et de discriminer des sites ayant des degrés de contaminations différents. Les organismes choisis apparaissent donc comme des

bioindicateurs efficaces pouvant être utilisés dans un programme de biosurveillance passive des contaminants.

Afin de mieux comprendre les dynamiques des processus d'accumulation et d'élimination des contaminants *in situ* et de déterminer si les organismes peuvent être utilisés dans un programme de biosurveillance active, des expériences de transplantation ont été réalisées. Tout d'abord, les algues *L. variegata,* les clams *G. tumidum* et les huîtres *I. isognomon* provenant de sites de « références » (baie Maa pour les huîtres et les algues, Plage d'Ouano pour les clams) ont été transplantés dans des sites très contaminés (baie de Boulari pour les huîtres et les algues, Grande Rade GR_I pour les clams) pour suivre l'accumulation des différents éléments en milieu naturel, et *vice versa* pour suivre l'élimination des éléments (***Chapitres 9 et 10***). Les résultats révèlent la forte capacité des organismes sélectionnés à bioaccumuler les éléments présents et à refléter le degré de contamination dû aux activités minières (Co, Cr, Mn et Ni) et aux activités urbaines (Ag) des organismes résidants dans les sites contaminés, tout particulièrement dans le cas de l'algue et du clam. Bien que surprenante, les résultats de la transplantation de clams de GR_I à la plage d'Ouano sont en adéquation avec les informations préalablement obtenus sur les concentrations d'As dans les populations natives de clams dans les différents sites, et confirment le potentiel important du clam transplanté à refléter l'état de contamination d'un site en As (***Chapitres 8 et 10***). Ces résultats démontrent notamment que l'utilisation combinée d'une biosurveillance passive à une biosurveillance active permet de déterminer si les teneurs en contaminants mesurés sont le reflet d'une contamination ancienne, ou reflètent au contraire une contamination actuelle. Dans le cas de l'As, nos résultats démontrent que cet élément est bien présent au site de la plage d'Ouano sous une forme hautement biodisponible pour le clam. Toutefois, à partir des données disponibles dans cette étude, il n'est pas possible d'identifier la source d'As (naturelle et/ou anthropique) à la plage d'Ouano. Bien qu'il soit généralement admis que la majeure partie de l'As est sous forme organique non toxique (e.g. arsénobétaïne, Francesconi & Edmonds 1998) dans les produits destinés à la consommation humaine, il n'existe actuellement aucune donnée concernant les formes physico-chimiques de l'As dans les tissus du clam *G. tumidum*, spéciation qui détermine sa toxicité (Kaise & Fukui 1992). L'As étant considéré comme un élément hautement toxique, une analyse des différentes formes chimiques de l'As dans les tissus du clam est fortement recommandée afin de s'assurer que la « grisette » ne présente pas de risque toxique pour le consommateur local.

Bien que les trois organismes sélectionnés présentent un fort potentiel de bioaccumulation *in situ* des éléments présents dans l'environnement marin, les expériences de transplantation montrent que leur utilisation en tant que bioindicateurs pour une biosurveillance active ou passive présente un certain nombre d'avantages mais aussi de limites dont il faut tenir compte. Alors que les algues *L. variegata* transplantées dans la baie de Boulari sont capables d'accumuler certains éléments (Co, Cr, Mn et Ni) à des concentrations qui excèdent celles des algues résidentes de la baie, dans certains cas, les bivalves transplantés dans un site contaminé n'atteignent pas les concentrations des organismes résidants dans les sites contaminés. Des résultats comparables ont été observées dans d'autres expériences de transplantation avec l'huître *Crassostrea rhizophorae* (Wallner-Kersanach et al. 2000), le clam *Macoma balthica* (Cain & Luoma 1985), la moule *M. edulis* (Gibb et al. 1996; Riget et al. 1997). Il se peut que les concentrations mesurées dans les organismes résidants dans les sites contaminés (Grande Rade GR_I, baie de Boulari) ne reflètent plus la contamination actuelle environnante, mais soit au contraire le reflet du passé historique des organismes en zone contaminée. Les espèces vivant au sein de biotopes naturellement riches en éléments métalliques peuvent avoir mis en place des mécanismes leur permettant de s'adapter aux fortes conditions contaminantes présentes dans la baie par une acclimatation physiologique ou une adaptation génétique (e.g. Klerks & Weis 1987; Ma et al. 2000). Cette acclimatation peut alors provoquer une modification du comportement bioaccumulateur (e.g. Wang & Rainbow 2005). La résistance aux contaminants peut se traduire (1) par une accumulation plus faible ou une excrétion plus forte des métaux (e.g. Bryan & Hummerstone 1973; Rainbow 2002) ou (2) par une augmentation de l'accumulation et/ou une meilleure capacité à séquestrer les contaminants sous une forme moins toxique (e.g. induction de protéines de détoxication, Roesijadi 1994; Wallace & Lopez 1997; piégeage dans des granules, Coombs & George 1978). Ainsi, bien que le suivi des contaminants dans les organismes résidents permette de déterminer l'évolution temporelle et spatiale des niveaux de contaminants dans certains sites, les niveaux mesurés ne reflètent pas forcément le degré de contamination réel. Par conséquent, les processus d'adaptation pouvant exister dans les populations résidentes pourraient être contournés à l'aide d'une biosurveillance active, ce qui faciliterait la comparaison des différentes stations et permettrait d'obtenir une meilleure représentativité de la contamination ambiante.

La transplantation d'organismes (algue, clam, huître) d'un milieu contaminé vers un milieu moins contaminé montre une rétention efficace des contaminants dans les organismes. Le Co

dans l'algue *L. variegata* et l'Ag, le Co et le Ni dans l'huître *I. isognomon* sont les seuls éléments présentant une élimination significative : leurs concentrations dans les organismes transplantés restent toujours supérieures à celles des organismes résidents des sites non contaminés. Une telle élimination partielle des éléments est souvent observée dans les expériences de transplantation (e.g. Zn dans la moule *Mytilus edulis,* Simpson 1979; Roesijadi et al. 1984; Cd et Cu dans l'huître *Crassostrea gigas*, Geffard et al. 2002; Zn, Cr et Cu dans le clam *Mercenaria mercenaria*, Behrens & Duedall 1981). La comparaison des temps de rétention obtenus sur le terrain et en laboratoire indique que les processus d'élimination *in situ* semblent plus lents que ceux observés en laboratoire. Wallner-Kersanach et al. (2000) ont observé que le Cu était plus facilement éliminé (30 % après 30 jours) dans les huîtres préalablement transplantées pendant 60 jours dans une zone fortement contaminée plutôt que dans les huîtres vivant naturellement dans la même zone contaminée (diminution de 9 % après 30 jours), lorsque les deux lots d'huîtres sont transplantés dans une zone propre. L'élimination des contaminants peut donc être influencée par le passé écotoxicologique des organismes vivants dans les zones contaminées, et des mécanismes d'adaptations permettraient d'expliquer la forte rétention observée *in situ* pour les éléments et les différences observées entre les résultats de laboratoire et *in situ*. Dans un contexte de biosurveillance, cette forte rétention des éléments présente l'avantage de préserver l'information relative à une contamination sur une longue période de temps, cependant elle peut entraîner certains problèmes. En effet (1) les concentrations mesurées à un temps donné dans des organismes transplantés peuvent ne pas refléter réellement la diminution des contaminants dans l'environnement sur de courtes périodes de temps (i.e. <3 mois) et (2) les concentrations mesurées dans les populations résidentes peuvent refléter une contamination ancienne et non plus nécessairement présente. Par conséquent afin de compléter les expériences de transplantation menées au cours de cette étude, il serait intéressant de déterminer l'influence du passé écotoxicologique sur la rétention des éléments et l'aptitude des organismes à refléter les diminutions de contaminants dans l'environnement en s'appuyant sur des protocoles expérimentaux semblables à ceux décrits par Wallner-Kersanach et al. (2000).

La transplantation de clams et d'huîtres provenant de sites non contaminés en Grande Rade dans les deux mêmes sites (GR1 et GR2) montre que l'accumulation des éléments s'est avérée plus rapide et plus efficace chez le clam que chez l'huître. Pourtant, les résultats obtenus en laboratoire ne laissaient pas présager une telle accumulation préférentielle pour le clam. En

effet, bien que ces deux bivalves aient des capacités d'accumulation relativement similaires des éléments liés aux sédiments, la capacité de l'huître à bioconcentrer les métaux dissouts est largement supérieure à celle du clam (jusqu'à plusieurs ordres de grandeurs). Par rapport aux conditions de laboratoire, où chaque voie d'accumulation est étudiée séparément, l'étude des cinétiques d'accumulation des contaminants dans les organismes *in situ* est plus difficilement interprétable en terme de sources d'accumulation, les contaminants étant présent sous différentes formes (e.g. dissoutes, particulaires). Cependant la comparaison des résultats obtenus en laboratoire et *in situ* suggère que la contribution de la voie dissoute, pourtant souvent considérée comme la voie majoritaire d'accumulation (e.g. Campbell 1995) dans la bioaccumulation globale des contaminants, aurait dans le cas du clam *G. tumidum* et de l'huître *I. isognomon* une contribution minoritaire par rapport à la voie particulaire. Ces observations sont semblables aux résultats de Luoma et al. (1992) et de Wang et al. (1996), pour lesquels la voie particulaire est la voie majoritaire d'accumulation pour le Se dans le clam *Macoma balthica* et la moule *Mytilus edulis* (96 - 99 %) et pour le Co (> 70 %), le Se (96 %) et le Zn (50 - 72 %) dans la moule *Mytilus edulis*. De tels résultats apportent des informations intéressantes en terme d'interprétation des données issues des programmes de biosurveillance, mais aussi en terme d'évaluation de la toxicité des contaminants pour les organismes, les tests basés sur la voie dissoute risquent de sous-estimer la toxicité des contaminants pour les organismes. Il serait dès lors nécessaire de connaître les concentrations dans le phytoplancton et dans les sédiments engendrant des effets biologiques/toxiques sur les bivalves lors d'une contamination par voie alimentaire et sédimentaire. La voie trophique est en effet généralement peu considérée dans les études toxicologiques ; or, si elle contribue pour une part importante à la bioaccumulation des contaminants dans les organismes (e.g. bivalves), la question est de savoir comment les informations relatives à la toxicité des contaminants dissouts sont extrapolables au milieu marin et aux normes de qualité de l'environnement ? La prise en compte d'autres compartiments de l'écosystème dans la détermination des normes de qualité pour l'environnement et dans l'évaluation de la toxicité des contaminants pour les espèces marines est un des pas à franchir afin de pouvoir mieux prédire les concentrations maximales tolérables dans le milieu marin et pour les organismes.

III. MISE EN PLACE D'UN PROGRAMME DE BIOSURVEILLANCE

Les expériences réalisées *in situ* dans cette étude confirment l'utilisation possible de *L. variegata G. tumidum* et *I. isognomon* dans deux types de stratégies de biosurveillance : passive et active. Dans la biosurveillance passive, le suivi des concentrations en contaminants utilise les populations résidentes d'organismes sauvages (e.g. Mussel Watch, Goldberg et al. 1983; RNO 2004, 2005) alors que la biosurveillance active a recourt aux transplants d'individus provenant d'un site de référence (RINBIO 2001). Le choix d'un biosurveillance active/passive réside essentiellement dans la présence/absence des espèces bioindicatrices dans les sites à surveiller. La biosurveillance active permet d'obtenir une manière directe d'évaluer les conditions d'exposition et présente trois avantages majeurs sur la traditionnelle surveillance des contaminants dans les populations naturelles (de Kock & Kramer 1994): (1) elle permet de réduire l'influence de nombreux facteurs externes et internes, susceptibles d'induire des fluctuations dans les mesures: variations saisonnières, variations pour une même classe d'âge ou de taille en raison de différences génétiques, (2) la période d'exposition dans un site contaminé est choisie et (3) les sites à surveiller peuvent être sélectionnés indépendamment de la présence de populations naturelles. Ce dernier point est en effet une des limitations principales des programmes de biosurveillance passive existant actuellement (e.g. Mussel Watch, Réseau National d'Observation-RNO-).

Les expériences de transplantations réalisées au cours de ce travail avec *L. variegata, G. tumidum,* et *I. isognomon* ont montré la faisabilité (facilité de manipulation, faible taux de mortalité) et l'utilité des organismes sélectionnés en tant que bioindicateurs fiables dans le cadre d'une biosurveillance active (***Chapitres 9 et 10***). De plus, de par les caractéristiques biologiques de ces trois espèces, différents biotopes pourront être suivis (milieu intertidal et/ou infratidal) ainsi que différentes sources potentielles de contamination (eau de mer, nourriture et sédiments). La biosurveillance active est utilisée pour surveiller un grand nombre de contaminants (e.g. PCB, Hummel et al. 1990; pesticides, Green et al. 1986; métaux, Mersch et al. 1996) et présente de nombreuses possibilités d'utilisation: le suivi de rejets (e.g. industriels, station d'épuration, rejets de dragage, rejets d'effluents), études d'impact, détection locale de contamination, surveillance globale de la qualité du milieu (e.g. Anderlini 1992; Smolders et al. 2003). Elle semble donc parfaitement adaptée à la surveillance des rejets de

l'effluent de l'usine Goro-Nickel dans le canal de la Havannah alors qu'une biosurveillance passive pourrait être mise en place relativement facilement dans les sites autour de la ville de Nouméa (e.g. Baie de Boulari, Grande Rade GR_I) afin de surveiller l'évolution temporelle de la contamination urbaine (e.g. Ag, Cu, Zn) et minière (e.g. Co, Cr, Mn, Ni). Ainsi, ces deux aspects de la biosurveillance vont être abordés dans les paragraphes suivants.

Pour la mise en place d'une biosurveillance des contaminants efficace et informative à l'aide de ces trois organismes, il est important de prendre en considération et de définir différents paramètres (e.g. la périodicité des prélèvements, les effectifs à considérer, les compartiments de l'écosystème à surveiller). Les paramètres des programmes de biosurveillance existant à l'heure actuelle (e.g. RNO, Mussel Watch, Réseau Intégrateurs Biologiques –RINBIO-) ont été pris en considération dans les propositions ci-dessous (Tableau 1), afin de proposer un programme de surveillance réalisable sur les côtes néo-calédoniennes.

Taille des organismes

Afin de réduire la variabilité des données, la taille est l'un des premiers facteurs à prendre en considération. Il est d'ailleurs recommandé d'utiliser des organismes avec une gamme de taille définie, > 35 mm pour les clams *G. tumidum* (***Chapitre 1***) et > 70 mm pour les huîtres *I. isognomon* (Metian 2003). Dans les programmes de biosurveillance (e.g. Mussel Watch, RINBIO), une gamme de taille des organismes est aussi sélectionnée (e.g. 5 à 8 cm pour les moules *M. edulis* et 7 à 10 cm pour les huîtres *C. virginica* et 2.5 à 5 cm pour *O. sandvicensis*, O'Connor 1998; 5 cm ± 0.5 pour les moules *M. galloprovincialis*, RINBIO 2001).

Saisons

L'influence des paramètres abiotiques (e.g. la saison, la température) sur la bioaccumulation des contaminants dans les organismes n'est pas directement contrôlable. Dans les programmes actuellement mis en place en milieu tempéré (e.g. RNO, Mussel Watch), la meilleure période de collection est la période hivernale (e.g. mi-Novembre à mi-Mars pour le Mussel Watch), lorsque les activités liées au cycle reproducteur sont faibles, permettant ainsi d'avoir une masse corporelle plus constante, et donc des concentrations métalliques peu influencés par l'activité sexuelle de l'organisme.

La biologie des espèces tropicales *G. tumidum* et *I. isognomon* a été beaucoup moins étudiée que celle d'autres espèces tempérées (e.g. *Mytilus edulis*) et il n'existe pas d'informations disponibles sur la période reproductive d'*I. isognomon* en Nouvelle-Calédonie. Cependant,

une étude de Baron (1992) concernant trois bivalves de Nouvelle Calédonie dont *G. tumidum* a montré le début de la maturation de ce clam à partir de 22 mm et l'existence d'un pic de reproduction entre Novembre et Mars, pendant l'été austral. Dans le cadre de la biosurveillance des côtes calédoniennes, il serait donc judicieux de collecter des organismes aux mêmes périodes chaque année et d'opérer pendant la phase de repos sexuel (avril-octobre) où le métabolisme des individus est plus stable.

Tableau 1. Comparaison entre le Réseau National d'Observation (RNO), le National Mussel Watch Project, intégré dans le National Status and Trends Program de la NOAA (National Oceanic and Atmospheric Administration) (NS&T) et le Réseau d'Intégrateurs Biologiques (RINBIO).

Données obtenus à partir de Beliaeff et al. 1998; Cantillo 1998; RNO 2005.

	RNO (crée en 1979)	**Mussel Watch NS&T (crée en 1986)**	**RINBIO (crée en 1994)**
	Ministère de l'environnement Coût annuel: 1.5MF (hors coordination)	Secrétariat du commerce Coût annuel: 2.5 MF (hors coordination)	
Nombre de coquillage par prélèvements	10 huîtres 50 moules	20 huîtres (3 triplicatas de 1986-1991, un après) 30 moules (3 triplicatas de 1986-1991, un après)	80 moules pour l'analyse chimique 15 moules pour le suivi biométrique
Espèces utilisées	*Crassostrea gigas* *Mytilus edulis* *M. galloprovincialis*	*Crassostrea virginica* *Mytilus edulis, M. californanus* (et d'autres espèces e.g. *C. rhizophorae, M. trossulus* et *M. galloprovincialis, Ostrea sandvicensis* –Hawaï-, *Dreissena polymorpha* – Grands Lacs-)	*M. galloprovincialis*
Contaminants mesurés dans la matière vivante*	Ag, Cd, Cr, Cu, Hg, Ni, Pb, V, Zn	Ag, Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb Se et Zn	As, Cd, Cr, Cu, Hg, Ni, Pb
Fréquence d'échantillonnage	- En métropole, deux fois par an (février et novembre) - Aux Antilles, 4 prélèvements par an (février, mai, août, novembre)	Avant 1991: Annuel Depuis 1992: Bisannuel	Tous les 2-3 ans
Nombre de points de prélèvements	90 dont 9 dans les DOM	89 (45 moules et 44 huîtres)	194 stations en 2003

* les composés organochlorés, Hydrocarbures Aromatiques Polycycliques HAP et radioéléments ne sont pas considérés dans cette présentation

Fréquence d'échantillonnage

L'objectif des programmes du RNO et du Mussel Watch est de suivre l'évolution temporelle et spatiale des contaminants ; ainsi, les échantillonnages s'effectuent une fois par an ou tous les deux ans. Dans le cadre néo-calédonien, l'objectif initial est de surveiller les rejets de l'effluent de la future usine de Goro-Nickel. De ce fait, un suivi plus régulier des niveaux de contaminants semble plus approprié afin d'être plus à même de déceler un problème de contamination liés aux rejets de l'usine. Par rapport aux capacités de bioaccumulation des organismes décrites en laboratoire (***Première Partie***) et *in situ* (***Deuxième Partie***), les prélèvements devraient être espacés d'au moins 2 mois pour les bivalves *G. tumidum* et *I. isognomon* et d'un mois pour les algues *L. variegata* pour que les organismes aient suffisamment de temps pour intégrer une éventuelle contamination dans leurs tissus.

Durée de la biosurveillance

Dans le cadre d'une biosurveillance passive, la durée du suivi n'est pas spécialement définie au départ, et peut varier en fonction des objectifs visés et des résultats obtenus. Un suivi sur une dizaine d'années est tout à fait envisageable, tout comme le montre les résultats du Mussel Watch (e.g. O'Connor 1998). Pour une biosurveillance active, la période de temps dépend du nombre d'organismes transplantés, de la fréquence d'échantillonnage et de l'objectif attendu (e.g. 4 à 5,5 mois, Smith et al. 1986; 2,5 mois, RINBIO 2001).

Choix des organismes

Lors de l'évaluation du niveau de contamination de l'environnement marin à l'aide de bioindicateurs, il est généralement recommandé d'utiliser simultanément plusieurs organismes sachant qu'il n'existe pas un bioindicateur universel (e.g. la moule *Mytilus edulis* et l'algue *Fucus serratus*, Gibb et al. 1996; une algue *Fucus vesiculosus*, un suspensivore *Mytilus edulis*, un déposivore *Macoma balthica* et un carnivore *Platichtys flesus*, Bryan et al. 1985). L'appartenance de nos trois espèces à des niches écologiques différentes permet de caractériser différentes fractions biodisponibles des contaminants présentes dans l'environnement. Dans le cadre de la surveillance des eaux néo-calédoniennes, l'utilisation simultanée d'indicateurs de métaux contenus dans la colonne d'eau (l'algue *L. variegata*) et dans la matière particulaire/dissoute (le clam *G. tumidum* et l'huître *I. isognomon*) permettrait d'évaluer le niveau de contamination environnemental sur des organismes appartenant à différentes niches écologiques et de prendre en compte les différences de bioaccumulation des espèces vis-à-vis des contaminants (Rainbow & Phillips 1993).

Nombre d'organismes

Dans une approche de biosurveillance, l'intérêt majeur est la détection d'une modification du statut de contamination d'un site via l'analyse des contaminants dans les organismes. Or la différence de concentration minimale détectable entre deux lots d'organismes va dépendre du nombre d'organismes analysés et de la variabilité des données (Gordon et al. 1980). Les résultats obtenus au cours de ce travail (***Chapitre 10***) montrent que l'utilisation de 50 à 60 clams ou huîtres permettrait de détecter des différences de concentrations appropriées entre deux lots d'organismes, par rapport aux différences naturelles mesurées dans le lagon de Nouvelle Calédonie. La sélection d'organismes avec une même gamme de taille et hors période de ponte permet de diminuer la variabilité inter-individuelle des données obtenues lors de la biosurveillance active/passive de la contamination. De plus, le prélèvement d'organismes provenant d'un même stock dans une station non contaminée, qui seront par la suite transplantés dans les sites à surveiller permet de réduire encore cette variabilité dans le cas d'une biosurveillance active. Toutefois, malgré une stratégie d'échantillonnage appropriée, un large degré de variabilité interindividuelle des concentrations métalliques persiste même à l'intérieur d'un même site (***Chapitre 10***), comme l'avaient observé précédemment Gordon et al. (1980) et Lobel et al. (1989). La détermination de la variabilité est une information importante permettant de mieux diagnostiquer les différences de concentrations entre deux lots d'organismes. Or de part le coût élevé des analyses de contaminants (particulièrement les contaminants organiques), l'analyse des contaminants dans les programmes de biosurveillance (e.g. Mussel Watch, RNO) se fait sur un/des pool(s) d'individus plutôt que sur chaque individu. Le programme de biosurveillance qui devrait être mise en place en Nouvelle Calédonie concernerait uniquement les métaux et métalloïdes, la partie analytique ne constituera donc pas le coût majeur du programme. Par conséquent, il est recommandé d'analyser les concentrations des contaminants dans chaque individu afin d'obtenir les informations relatives à la variabilité inter-individuelle.

En résumé et pour conclure, l'ensemble de ce travail a apporté des informations nouvelles et originales sur les mécanismes de bioaccumulation des contaminants d'importance majeure (Co, Cr, Mn, Ni) et secondaire (Ag, As, Cd, Cu, Zn) en Nouvelle Calédonie dans les espèces tropicales *L. variegata*, *G. tumidum* et *I. isognomon*. Ces informations constituent la base de la mise en place d'un système de biosurveillance de la contamination des eaux calédoniennes par l'utilisation de ces espèces tropicales appropriées

aux côtes calédoniennes, dans une double approche, active et passive. L'utilisation d'une biosurveillance active et passive sur les côtes calédoniennes permettrait d'évaluer la contamination métallique existante et de suivre son évolution au cours du temps, et d'évaluer le risque pour la santé humaine pour ce qui concerne les espèces consommées. La large répartition géographique de l'algue *L. variegata* et du clam *G. tumidum* dans la province indopacifique (e.g. *L. variegata* à Hawaii, *G. tumidum* au Japon et aux Philippines) associé à leur fort potentiel en tant qu'espèces bioindicatrices montrent que non seulement *L. variegata* et *G. tumidum* peuvent être proposés pour un programme de biosurveillance des côtes calédoniennes, mais que leur utilisation pourrait être également étendue à d'autres zones tropicales où ces espèces sont présentes.

Concernant le devenir des contaminants, il est important de déterminer comment la bioaccumulation conditionne les effets des métaux potentiellement toxiques vis-à-vis des organismes, et notamment des échelons trophiques supérieurs. Bien que le lien entre la bioaccumulation et la toxicité des métaux dans les organismes sélectionnés ne soit pas abordé dans cette étude, il constitue une des perspectives importantes à prendre en considération, notamment pour le clam, consommée par les populations locales, mais aussi pour d'autres espèces tropicales plus sensibles aux contaminants. S'il existe de nombreux travaux concernant les tests de toxicité en milieu tempéré, force est de constater que le domaine de l'écotoxicologie tropicale est peu exploité et des tests spécifiques applicables restent encore à définir. En effet, bien que la toxicité chronique du lixiviat de résidus ait été évaluée via des tests normalisés de fécondation de l'oursin *Heliocidaris tuberculata*, d'anomalies du développement embryo-larvaire de la Coquille St Jacques *Mimachlamys asperrima* et de l'huître *Saccostrea commercialis*, et d'inhibition de la croissance de *Nitzschia closterium* (e.g. Spencer et al. 1999; Stauber et al. 2003), parmi les espèces utilisées, seule la microalgue *Nitzschia closterium* est susceptible d'exister dans les eaux côtières de la Nouvelle Calédonie. Ainsi, le développement de protocoles d'essais écotoxicologiques chroniques sur des organismes locaux est un des challenges majeurs à mettre en œuvre pour une prédiction efficace et réaliste de l'impact des contaminants sur l'environnement tropical de Nouvelle-Calédonie. De plus, bien qu'historiquement, les tests toxicologiques se soient focalisés sur les effets des concentrations en contaminants dissouts, un intérêt croissant est aujourd'hui porté à la toxicité des sédiments. Les contaminants présents dans les sédiments peuvent avoir des effets néfastes sur les organismes aquatiques et sur les populations consommant les ressources aquatiques (Long 2000; McCauley et al. 2000). Des critères de concentrations en

contaminants dans les sédiments (concentrations ERL -Effects Range-Low- et ERM -Effects Range-Median -, Guide pour la Qualité des Sédiments, Long et al. 1995; NOAA 1999) ont récemment été développés par les autorités américaines à partir d'expériences de laboratoire et de terrain en Amérique du Nord. Ces critères déterminent les concentrations pour lesquelles des effets biologiques sont rarement (< ERL), occasionnellement (compris entre ERL et ERM) ou fréquemment (> ERM) rencontrés. Selon ces critères, la Grande Rade, la baie de Boulari, la baie de Dumbéa et la baie de Sainte-Marie peuvent être considérées comme significativement polluées en Ni, Cr et Zn (Galindo Dalto 2005). Notre étude suggérant que la voie particulaire pourrait jouer un rôle important dans la bioaccumulation globale des contaminants dans les organismes, et les caractéristiques géochimiques des sédiments étant connus pour modifier la bioaccumulation des contaminants (e.g. Gagnon & Fisher 1997), une des perspective intéressante de cette étude serait tout d'abord d'évaluer la qualité des sédiments de Nouvelle-Calédonie pour y adapter les seuils de toxicité développés dans le Guide pour la qualité des sédiments. Le domaine des tests de toxicité en milieu tropical est un secteur à développer rapidement afin de disposer de tests utilisant des espèces locales et prenant en compte la voie sédimentaire et la voie trophique, ceci afin d'évaluer au mieux les critères de la qualité environnementale et la nocivité de l'effluent pour les espèces marines locales, et surtout de permettre la mise en place de mesure permettant de protéger le plus efficacement possible les écosystèmes tropicaux.

Les travaux présentés ici se sont focalisés sur le développement de bioindicateurs permettant la caractérisation de l'ampleur de la contamination afin de surveiller à terme les tendances temporelles et spatiales des contaminants le long des côtes calédoniennes en utilisant *L. variegata*, *G. tumidum* et *I. isognomon* Toutefois, ce type de surveillance ne donne pas d'informations sur les effets des contaminants sur les organismes, et les écosystèmes. Le développement de méthodes plus sensibles et plus rapides permettant de mesurer les effets biologiques des contaminants, tels que les biomarqueurs (e.g. métallothionéines, fragilité lysosomale, activité de l'acétylcholinestérase) permettant de mettre en évidence de façon précoce les effets des contaminants sur les organismes, est aujourd'hui la prochaine étape à franchir. L'utilisation des biomarqueurs conjointement à une surveillance de l'ampleur de la contamination par les bioindicateurs développés dans cette étude permettraient de mieux comprendre les relations entre la présence d'un contaminant et ses effets sur l'environnement, et de disposer d'outils permettant non seulement de déceler la présence de contaminants mais aussi de prédire les effets d'une contamination sur les écosystèmes (e.g. apparition de

maladies…). L'utilisation combinée de biomarqueurs et de bioindicateurs en tant qu'outils de prédiction du risque écologique des contaminants permettraient dès lors de prendre les mesures de protection nécessaires (e.g. initiation de stratégies de « bioremédiation) » avant que des dommages environnementaux irréversibles liés à la contamination se soient totalement installés.

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ANNEXES

I. ANNEXE 1

Table 1. Localisation des stations de récoltes et de transplantation de *Malleus regula, Isognomon isognomon, Lobophora variegata* et *Gafrarium tumidum.*

Site	Localisation		Espèces récoltées
	Latitude	Longitude	
Sainte-Marie	22°18'55 S	166°27'98 E	*I. isognomon, M. regula, L. variegata*
Koutio	22°13'47 S	166°25'32 E	*I. isognomon, M. regula, L. variegata*
Boulari	22°17'37 S	166°31'89 E	*I. isognomon, M. regula, L. variegata*
Maa	22°12'29 S	166°19'42 E	*I. isognomon, M. regula, L. variegata*
Grande Rade -GR_S-	22°15'00 S	166°23'94 E	*I. isognomon*
Dumbéa	22°11'25 S	166°24'38 E	*G. tumidum*
Grande Rade -GR_I-	22°15'09 S	166°26'28 E	*G. tumidum*
Plage d'Ouano	21°52'06 S	165°48'92 E	*G. tumidum*
Grande Rade -GR1-*	22°15'42 S	166°26'06 E	
Grande Rade -GR2-*	22°15'22 S	166°24'20 E	

* site de transplantation

II. ANNEXE 2

Use of Radiotracer Techniques to Study Subcellular Distribution of Metals and Radionuclides in Bivalves from the Nouméa Lagoon, New Caledonia

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I. INTRODUCTION

New Caledonia is the third largest producer of nickel in the world, and this small South Pacific island is estimated to contain no less than 20% of the total stock of Ni on the planet (Connell 2003). Metal contamination resulting from the nickel mining industry and related activities constitutes a long lasting threat to the marine ecosystems sheltered by the second largest reef system in the world (Labrosse et al. 2000). However, as almost a rule when it concerns tropical ecotoxicology, available information on metal contamination in New Caledonia waters is extremely scarce and very little is known about the extent of local contamination and possible environmental impacts (Labrosse et al. 2000). Moreover, a new extraction process for Ni ("lixiviation", *viz.* acidic extraction) has recently been tested at the industrial level and should be implemented in the near future (2006-2007). This process will result inevitably in increased discharges of co-occurring metals in Ni ores (e.g. Co and Cr). Thus, basic information on metal metabolism and behaviour is needed in order to assess the possible impact of these additional metal inputs on local ecosystems.

The objective of the present study was to determine the potential toxicity of metals in two species commonly found in the lagoon: the edible clam *Gafrarium tumidum* and the oyster *Isognomon isognomon*. Contaminant partitioning within the cells (soluble vs. insoluble fractions) determines the likelihood of inducing deleterious effects (reaction with cellular components) (Viarengo 1985) as well as to being transferred to higher trophic levels (Reinfelder & Fisher 1991). Therefore, the subcellular distribution of five metals (Cd, Co, Cr, Zn, Ag) and two anthropogenic radionuclides (^{134}Cs, ^{241}Am) was examined in the gills and visceral mass of both species following direct seawater exposure to these elements using highly sensitive radiotracer techniques.

II. MATERIALS AND METHODS

Both bivalve species were collected in August 2002 by SCUBA diving in Dumbéa bay (*G. tumidum*) and Maa bay (*I. isognomon*) (Nouméa, New Caledonia) and were immediately shipped to the IAEA-MEL premises in Monaco where they were

acclimated to laboratory conditions (open circuit aquaria; water renewal 10% hr^{-1}; S: 36 p.s.u.; T: 25±0.5°C) for 6 wk prior to experimentation.

The organisms were then experimentally exposed for 28 d to radiotracers of five heavy metals (^{109}Cd, ^{57}Co, ^{51}Cr, ^{65}Zn, ^{110m}Ag) and two radionuclides (^{134}Cs, ^{241}Am) directly via sea water. Periodically during the exposure phase, the bivalves were transferred to unlabelled sea water for a short time (1-2 hr) where they fed on mixed phytoplankton cultures before being returned to the labelled sea water for further uptake. At the end of the experiment, 6 individuals of each species were collected and dissected. The gills and visceral mass were separated, pooled, and processed for subcellular fractioning, using differential centrifugation (Galey et al. 1983; Milcent et al. 1996). Briefly, homogeneized tissues were centrifuged successively:

- at 900 × *g* for 10 min (to sediment nuclei and heavy lysosomes),

- at 12,000 × *g* for 15 min (to sediment lysosomes and mitochondria),

- at 45,000 × *g* for 30 min (to sediment light mitochondria and plasma membranes),

- and finally at 115,000 × *g* for 70 min (to separate microsomes from the cytosolic fraction, the latter constituting the supernatant).

Distribution of the metal radiotracers and radionuclides among the different subcellular fractions was determined using a high-resolution γ-spectrometry system consisting of 4 coaxial Ge (N- or P-type) detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyzer and a computer equipped with a spectra analysis software (Interwinner, Intertechnique). The detectors were calibrated with appropriate standards for the counting geometry used, and all measurements were corrected for background and physical decay of the radiotracers. Counting times were adapted to obtain count rates with relative propagated errors less than 5%.

III. RESULTS AND DISCUSSION

Measurements of specific enzymatic markers (acid phosphatase for lysosomes, glucose-6-phosphatase for microsomes, 5'-nucleotidase for plasma membranes [Galey et al. 1983; Milcent et al. 1996]) indicated that the purity of the different subcellular fractions was good. Results of the subcellular distribution of the different metal radiotracers and radionuclides in gills and visceral mass are given in Table 1.

Table 1. Subcellular partitioning (mean %) of radioisotopes in gills and visceral mass of two bivalves.

	Gafrarium tumidum							*Isognomon isognomon*						
	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag	^{134}Cs	^{241}Am	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag	^{134}Cs	^{241}Am
1- GILLS														
Nuclei	18	28	28	30	73	20	25	17	16	22	14	23	17	27
Lysosomes + mitochondria	6	7	6	2	6	7	6	10	19	30	15	34	12	36
Membranes	10	17	16	1	6	13	25	19	8	15	10	23	16	10
Microsomes	10	22	19	1	5	13	27	10	5	0	6	7	11	4
Cytosol	57	25	31	67	10	48	17	44	52	33	54	13	45	22
2- VISCERAL MASS														
Nuclei	28	9	24	10	49	27	42	25	20	28	24	43	26	35
Lysosomes + mitochondria	13	6	10	2	12	11	22	22	10	20	15	27	19	47
Membranes	7	3	15	1	3	6	19	6	2	5	3	19	6	6
Microsomes	6	3	12	1	2	5	13	7	3	6	3	4	7	3
Cytosol	45	79	40	87	35	51	4	39	65	41	54	7	42	9

Globally, the distributions in both tissues were similar for each bivalve species. The only main departure from this was observed for ^{57}Co in the clam where the cytosolic fraction was much lower in the gills (25%) than in the visceral mass (79%). Cr, Co, Zn, Cd and ^{134}Cs were mainly found in the cytosolic fraction (30-87%) whereas ^{110m}Ag and ^{241}Am were mainly associated with membranes and organelles (65–96%). These results are in agreement with those reported for other bivalves from temperate waters, e.g., the scallop *Chlamys varia* (Bustamante & Miramand 2005) and the oyster *Crassostrea gigas* (Milcent et al. 1996).

The predominant distribution of Ag in the insoluble fraction (*viz.* the non-cytosolic fractions) could be due to specific Ag storage/detoxification in these two bivalve species. Indeed, it is well documented that various bivalves are able to trap Ag as non-toxic Ag_2S precipitates within their tissues (Berthet et al. 1990; Berthet et al. 1992). This kind of sequestration can inhibit the deleterious effects that could be caused by this highly toxic element, even if present in high concentrations. In addition, preferential distribution in the insoluble subcellular fraction indicates that Ag is not likely to be bioavailable to higher trophic levels (Bustamante & Miramand 2005).

Preferential distribution of most radioelements in the cytosol suggests that, once incorporated into the cells, a large part of these metals could be toxic, since they are likely to bind with key soluble components of the cells (e.g. proteins, enzymes, DNA). However, in the case of Cd and Zn, a substantial fraction of the cytosolic metal is most probably detoxified as “metal-metalloprotein” complexes, e.g. approximately 40% in the case of Cd in oysters (Boisson et al. 2003). Furthermore, the metals preferentially associated with the cytosolic fraction are likely to be readily bioavailable to higher trophic levels preying on these organisms (Reinfelder & Fisher 1991). This fact is of particular concern here since the clam *G. tumidum* is consumed by local populations, and could therefore be a non-negligible source of human exposure to metals through seafood consumption.

Progress in Ni ore exploitation planned in New Caledonia will result in an increased input of dissolved metals to New Caledonian lagoon waters (Morreton et al. 2004). Such a situation could result in an increased contamination of the local bivalves. Our findings indicate that subcellular partitioning of metals co-occurring in Ni ores will be preferentially cytosolic. Therefore, metal exposure of organisms (including man) preying on these two bivalves could be enhanced as well. Monitoring of metal contamination levels in edible species is therefore recommended following the industrial implementation of the acidic lixiviation process in New Caledonia.

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RESUME : CARACTERISATION D'ESPECES BIOINDICATRICES POUR LA SURVEILLANCE DES ACTIVITES MINIERES ET LA GESTION DE L'ENVIRONNEMENT EN MILIEU RECIFAL ET LAGONAIRE : APPLICATION AU LAGON DE NOUVELLE-CALEDONIE

Les activités minières constituent la principale ressource économique de la Nouvelle-Calédonie. La présente recherche doctorale a été réalisée afin de développer un système de surveillance de la contamination minière des eaux lagonaires au moyen d'organismes marins « bioindicateurs » : l'algue brune *Lobophora variegata,* les huîtres *Malleus regula* et *Isognomon isognomon* et le clam *Gafrarium tumidum.* Dans ce contexte, la valeur bioindicative de ces espèces vis-à-vis de l'Ag, As, Cd, Co, Cr, Mn, Ni et Zn a été déterminée en laboratoire et *in situ.*

L'étude en laboratoire du comportement bioaccumulateur des quatre organismes à l'aide des techniques de radiotraçage a démontré leur importante capacité de bioaccumulation et de rétention des contaminants sélectionnés. De plus, ces organismes répondent au principal critère que doit rencontrer un bioindicateur : les concentrations mesurées dans les tissus reflètent celles qui sont présentes dans l'environnement.

Dans les expériences *in situ*, la mesure des concentrations corporelles en contaminants dans les populations résidentes de clams, d'huîtres et d'algues a permis de discriminer des sites selon leur degré de contamination. De plus, la transplantation d'organismes entre des sites propres et contaminés (et *vice versa*) a montré que les espèces sont capables de refléter le degré de contamination des sites et qu'elles peuvent être utilisées pour surveiller des sites où les espèces ne sont pas naturellement présentes.

Ainsi, *L. variegata, G. tumidum* et *I. isognomon* sont des bioindicateurs fiables et très prometteurs qui peuvent être utilisés pour la biosurveillance passive et active de la contamination du lagon de Nouvelle-Calédonie.

MOT-CLES : Nouvelle-Calédonie, Bioindicateur, Activités Minières, Métal, Nickel, Bivalves, Algues, Radiotraceur

ABSTRACT: CHARACTERISATION OF BIOINDICATOR SPECIES FOR THE MONITORING OF MINING ACTIVITIES AND THE ENVIRONMENTAL MANAGEMENT OF CORAL REEF ECOSYSTEMS: APPLICATION TO THE NEW CALEDONIA LAGOON

Mining activities constitute the major economic resource of New Caledonia. This doctoral research was realized in order to develop a programme for biomonitoring mining contamination in the New Caledonia coastal waters using marine organisms as "bioindicators": the brown alga *Lobophora variegata*, the oysters *Malleus regula* and *Isognomon isognomon* and the clam *Gafrarium tumidum*. In this context, the bioindicative value of these four organisms for Ag, As, Cd, Co, Cr, Mn, Ni and Zn has been investigated through both laboratory and field experiments.

Laboratory investigations of the bioaccumulation behaviour of the four organisms using radiotracer techniques demonstrated their high bioaccumulation and retention capacities for the contaminants tested. Moreover, the organisms respond to the most relevant criteria of a bioindicator species: the contaminant concentrations in organisms actually reflect those occurring in the environment.

Field experiments showed that the analysis of contaminant concentrations in resident populations of clams, oysters and algae allowed discriminating sites according to their degree of contamination. In addition, transplantations of organisms between clean and contaminated sites indicated that the species displayed efficient bioaccumulation capacities for the contaminants *in situ*, and consequently, they can be used to monitor sites where the species were not naturally present.

Overall, it is concluded that *L. variegata, G. tumidum* and *I. isognomon* are efficient and reliable bioindicator species that may be used for active and passive biomonitoring of mining contamination in the lagoon of New Caledonia.

KEYWORDS : New Caledonia, Bioindicator, Mining Activities, Metal, Nickel, Bivalves, Algae, Radiotracer